CLINICAL AND METABOLIC EFFECTS OF THE MENOPAUSE AND THE ROLE OF REPLACEMENT OESTROGEN THERAPY

THESIS PRESENTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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by

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SUMMARY OF CONTRIBUTION TO KNOWLEDGE OF THIS INVESTIGATION

The present investigation was undertaken in order to clarify the clinical and metabolic effects of the menopause following bilateral oophorectomy in the human female and to evaluate the role of subsequent replacement exogenous oestrogen therapy. To this end pertinent clinical and laboratory studies were carried out. It is considered that the following contributions to knowledge have been made :

1. The symptoms and clinical signs directly related to endogenous oestrogen withdrawal following bilateral oophorectomy have been clarified and defined.
2. Symptoms responding to exogenous oestrogen therapy have been differentiated from those responding to placebo (general supportive) therapy.
3. The ability of exogenous oestrogens to produce a feeling of well-being in postmenopausal females is statistically substantiated.
4. The parabasal cell is shown to be the best cytologic index of the oestrogenic status of the postmenopausal female. Claims for an increase of vaginal superficial cells with exogenous oestrogen therapy are refuted.
5. Bilateral oophorectomy in the female of reproductive age is shown to have no effect for up to 2 years on the total serum cholesterol level. Oestrogen therapy, however, reduced the level of serum cholesterol in oophorectomized females.
6. The effects of bilateral oophorectomy on plasma calcium and inorganic phosphorus are shown. The ability of oestrogen to lower plasma calcium and phosphorus is demonstrated and the possible explanation and implication of these findings is discussed.

7. A controlled comparative evaluation of two different forms of oestrogen demonstrates different oestrogens to have different effects. Moreover, endogenous and exogenous oestrogens are shown to be not entirely similar in their clinical and metabolic effects.
8. The specific short-term sequelae of oophorectomy are demonstrated and the current role of exogenous oestrogen replacement therapy in postmenopausal females is discussed.

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PART I

INTRODUCTION AND REVIEW OF THE LITERATURE

1.1 INTRODUCTION

There is a paucity of established scientific data relating to the human climacteric, whether natural or induced by surgical bilateral oophorectomy (castration).

The 'climacteric' is the counterpart of puberty and is the transitory phase between sexual maturity and old age. The 'menopause' refers only to cessation of menstruation and occurs at a point of time during the climacteric. The years preceding the menopause may be called the 'premenopause' and the years following it the 'postmenopause'. The menopause is merely one manifestation of the climacteric and precedes complete cessation of ovarian function by several months or years (Jeffcoate, 1967). The 'senium' is the stage of life characterised by the gradual transition into old age (senility). Although common usage tends to equate the terms 'climacteric' and 'menopause' the strict definition of these will be adhered to in the present thesis (Kaiser and Daume, 1965).

Notable deficiencies in the English medical literature at the time of initiation of this thesis were as follows :

1. The real clinical and metabolic changes related specifically to oestrogen withdrawal at the climacteric or following bilateral oophorectomy.
2. The short, medium and long term effects of bilateral oophorectomy on the pre- and postmenopausal female.
3. The effect of exogenous oestrogen administration on such patients.
4. The relationship between exogenous (therapeutic) oestrogens and endogenous (ovarian) oestrogens and the question as to whether their effects on the human female are alike or differ.

5. Comparative clinical trials, statistically analysed, between different forms of exogenous oestrogen.
6. The relationship between therapeutic oestrogen effects and placebo effects.

Two further considerations influenced the author to undertake the present study. The first was the increasing number of women undergoing surgical castration, often as an adjunct to other gynaecological surgery or as a therapeutic measure for carcinoma of the breast; the second was the recent tendency to prescribe oestrogens to all postmenopausal women as a panacea against ageing. It was therefore considered necessary that a broad approach to the above problems be made.

Accordingly, a clinical research project taking such factors into account was planned and initiated. Several aspects of this investigation have been satisfactorily studied by other authors. The project as a whole, however, is considered unique in that, to the best of the author's knowledge, no other investigation exists in which so many clinical and biological parameters have been measured at the same time in an identical experimental population comprising such varying control groups and which have then been subjected to meticulous statistical analysis.

This thesis is based on the results of the above clinical research and was undertaken during the tenure of a full-time Lecturer post in the Department of Gynaecology, University of Cape Town and the Groote Schuur Hospital, Cape Town.

1.2 HISTORICAL BACKGROUND

1.2.1 CLIMACTERIC (MENOPAUSE)

The prevailing view of the menopause until the turn of the nineteenth century was one of despair, as evidenced by the following quotation from Colombat's chapter on the 'Change of Life' (1850) :

> 'Compelled to yield to the power of time, women now cease to exist as for the species, and henceforward live only for themselves. Their features are stamped with the impress of age, and their genital organs are sealed with the signet of sterility..... It is the dictate of prudence to avoid all such circumstances as might tend to awaken any erotic thoughts in the mind and reanimate a sentiment that ought rather to become extinct ..... in fine, everything calculated to cause regret for charms that are lost, and enjoyments that are ended forever'.

Fortunately a more modern view of the problem of the menopause began in the last decade of the nineteenth century, from which time reports of physiological, pathological and mental changes started to appear in the literature.

1.2.2 SURGICAL REMOVAL OF OVARIES

In 1685 Justice Theodorus Schorkopff published the earliest feature devoted entirely to ovarian cysts and suggested extirpation as a cure - 'provided' he added 'the operation were not too cruel and hazardous'. He did not, however, perform the operation and the contributions of Ephraim McDowell (1771 - 1830) form the fundamental knowledge upon which abdominal gynaecological surgery rests. The year 1809 marked an important advance in gynaecological therapy and, indeed, a milestone in abdominal surgery. In that year McDowell, after fourteen years of practice in the frontier town of Danville, Kentucky, successfully performed the first ovariotomy. He fully realized that the procedure was an experimental one and the first report

only appeared in 1816. For many years the profession in general failed to give approval and to Sir Thomas Spencer Wells (1818 - 1897) must be 'accorded the credit of having placed ovariotomy in the position not only of an acknowledged operation, but one of the most successful of one of the great operations of surgery' (Lawson Tait). Charles Clay (1801 - 1893) may be called the 'father of ovariotomy' in Britain because he performed the first operation in 1842 (Kerr, J.M. Munro et al, 1954). As time went on he did so many of these operations that he used to reckon his performances by the ton, thinking nothing of referring to 2,000 pounds by weight removal of ovarian tumours per month.

Robert Battey (1828 - 1895) was the first to suggest the operation of oophorectomy for such conditions as dysmenorrhoea and neuroses. Battey performed the first oophorectomy on August 27, 1872. He attempted to justify the operation of removal of normal ovaries by stating 'the removal of both ovaries puts an end to ovulation entirely and this determines the menopause or change of life; whereby I have hoped, through the intervention of the great nervous revolution which ordinarily accompanies the climacteric, to uproot and remove serious sexual disorders and re-establish the general health'. He did qualify this further, perhaps somewhat prophetically, by stating that 'I believe these organs should alone be sacrificed for grave causes, and then only as a dernier resort, when the hitherto recognized resources of our art have been expended in vain'.

Since the above early development the operation of bilateral oophorectomy has become a routine practice at hysterectomy performed by some gynaecologists. The ostensible reason is to prevent the subsequent development of ovarian malignancy. Heated controversy exists as to the justification for the procedure, as

evidenced by the enormous literature that has accumulated and which is reviewed under 1.5.

1.2.3 OVARIAN (ORGANO) THERAPY

Organotherapy or glandular therapy was a very ancient form of treatment (Ricci, 1945 and 1950). The Egyptians were known to eat the penis of an ass as a cure for impotence. The Greeks and Romans prescribed the testicles of the ass for this purpose. At the age of 72, Brown-Sequard (1889) reported before the Societé de Biologie of Paris (June 1, 1888) that he had rejuvenated himself by injections of 'testicular juice' The improvement in health manifested itself in greater body vigour, better vesical (sphincteric) action and better intestinal activity. According to Brown-Sequard, Augusta Brown used testicular extract to combat feminine debility.

At the close of the nineteenth century ovarian therapy was limited to the administration of crude ovaries, ovarian juice (suc ovarien), powdered ovaries and powdered ovarian tablets. These substances were used for conditions such as physiological and surgical menopause, dysmenorrhoea and adiposity (Ricci, 1945).

1.2.4 THE DEVELOPMENT OF OESTROGENIC HORMONES

It is generally accepted that the first genuine belief in the existence of an 'internal secretion' was voiced by Théophile de Bordeu in 1775. The actual term 'internal secretion' was first employed by Claude Bernard in 1855 in a lecture at the Collége de France (Kerr, J.M. Munro et al, 1954). Early this century William Baylis and E.H. Starling were dissatisfied with the term 'internal secretion' and in search of a better consulted various people, including William Hardy who, after discussion with W.T. Vesey of Cambridge suggested the word 'Hormone' (Kerr, J.M. Munro et al, 1954).

In 1849 came the first factual discovery of the physiology of the sex organs and their hormones when A.A. Berthold showed that the transplantation of cocks' testes into some other part of the body prevented the atrophy of the cock's comb which usually followed castration. He attributed this result to the influence exerted by the testes on the blood and thence on the body as a whole.

It was not until the present century that the modern conception of ovarian physiology took shape. In 1912 Adler produced the changes of oestrus by injecting into virgin animals watery extracts of ovary. Estimation of oestrogenic effect on vaginal smears is based on an observation by Stockard and Papanicolaou (1917) that the vaginal epithelium of rodents becomes cornified at the onset of oestrus. The reaction occurs not only in mature animals but also in immature and ovariectomized animals. It was the recognition of this last fact by Allen and Doisy in 1924 that gave origin to their test for the assessment of the activity of oestrogens (Kerr et al, 1954). Oestrin was the name given in 1926 by Parkes and Bellerby to the hormone extracted from the ovary by fat solvents.

Butenandt, a Nobel Prize winner for chemistry, succeeded with other research workers in 1929 in isolating and obtaining in pure form a hormone from the urine of pregnant women which was eventually called oestrone. Somewhat later oestriol was discovered in human pregnancy urine. The structural formulae of these hormones was worked out by Butenandt (1930) and others. It was not until 1940 that the presence of B-oestradiol was demonstrated in human pregnancy urine and in the placenta. Oestradiol is the most effective oestrogen in the female that is known today.

1.3 CLINICAL EFFECTS OF THE CLIMACTERIC

There is virtually no published work relating specific symptoms or signs with oestrogen production or excretion. Hence the evaluation and precise assessment of clinical features thought to be entirely a result of the climacteric is extremely difficult. Thus, the majority of clinical features ascribed to this non-specific period in the human life cycle are merely assumptions and may be no more than coincidental features in a generally ageing population (Papanicolaou et al, 1969a).

The only known factors governing the age of the menopause are familial, racial and possibly socio-economic status. The menopause usually occurs between the ages of 45 and 50 years with an average of 47 years in Great Britain and the United States of America (Jeffcoate, 1967). The mean age of the menopause appears to be rising (Frommer, 1964). No statistics are available for the South African population.

There is in the literature little information concerning the number of women who have sufficient symptoms at the time of the menopause to seek medical advice. A statistical study of 1,000 women in England indicated that 15.8 per cent had no symptoms at the time of the menopause, 62.3 per cent had only hot flushes for an average duration of two years and 89.7 per cent carried on their daily activities without interruption (Sub-Committee of Council of Medical Women's Federation of England, 1933). Although some reported that as many as 75 per cent of women have distressful symptoms at the menopause (Hawkinson, 1938; Wilson, R.A. and Wilson, T.A., 1963) it is more generally agreed that 10 to 15 per cent of women will present themselves for medical advice for symptoms attributed to the climacteric (Novak, 1954; Newton and Odom, 1964).

Furthermore, the nature and incidence of symptomatology varies in different educational, racial and population groups (Rogers, 1956 a; Newton and Odom, 1964). Any investigation into the climacteric therefore needs meticulous selection of clinical material.

The one definite feature of the climacteric is the menopause. Premenopausal symptoms never occur, according to Jeffcoate (1967) for the reason that the ovary continues to function, although to a lesser extent, until some time after the menopause. Other workers do not agree with this viewpoint, although the only premenopausal symptom they describe in fact relates to an alteration in the menstrual cycle (irregularity, increase in length of cycle or decrease in flow) (Newton and Odom, 1964; Rogers, 1956 a; Wilson and Wilson, 1963).

The symptoms which arise at the menopause are often of a psychological nature (Forman, 1968); in addition, there is frequently evidence of vasomotor instability. Many of the clinical features are believed to result from a decline and eventual failure of ovarian function. This is discussed further under 1.4.1. For convenience the symptomatology and untoward effects of the climacteric are usually classified, albeit artificially, into 3 groups as follows :

AUTONOMIC NERVOUS SYSTEM IMBALANCE	PSYCHOGENIC	FEATURES RELATED TO METABOLIC CHANGES
Hot flushes	Headaches	Senile vaginitis
Perspiration	Insomnia	Atheromatosis and Thrombosis
Palpitations	Mood changes	Skin atrophy
Angina pectoris	Irritability	Breast atrophy
	Depression	Osteoporosis
	Frigidity	Degenerative arthropathy
	Apprehension	Hirsuties

It is most likely that the only actual symptoms resulting from diminished ovarian activity are amenorrhoea and vasomotor instability as manifested by hot flushes (Rogers, 1956 a). The mechanism of the hot flush was studied by Reynolds et al (1941 a, 1941 b). The flush is usually described as a sudden feeling of heat in the face, neck and chest, associated with diffuse or patchy flushing of the skin or perspiration. Vasodilatation is followed by vasoconstriction, so after a flush comes a cold shiver. The flush is a manifestation of vasomotor instability and is brought on particularly by excitement or nervousness. The hot flush is the one symptom allowing of definite measurement in that the patient is usually able to describe the number of hot flushes suffered per twenty-four hour period.

The host of other symptoms such as palpitations, angina pectoris, headaches, irritability, depression, frigidity and apprehension that are listed as components of the 'menopausal syndrome' (Rogers, 1956 a) are probably manifestations of the psychological disturbances that may occur at this time of life and there is no evidence that these symptoms are causally related to oestrogen lack (Forman, 1968; Greenblatt, 1963; McCandless, 1964; Newton and Odom, 1964; Rogers, 1956 a; Squires and Cannell, 1952; Young, 1939).

Symptoms and signs may be related to metabolic changes. Thus atrophic (senile) vaginitis, resulting directly from oestrogen deficiency may produce symptoms of dyspareunia or pruritus vulvae (Jeffcoate, 1967; Rogers, 1956 a). Furthermore, oestrogen deprivation appears to be associated with breast atrophy and possibly skin atrophy (Greenblatt, 1963; Jeffcoate, 1967; Kretzshmar, 1964; Wilson, R.A. and Wilson, T.A., 1963). The clinical and metabolic aspects of the locomotor and cardio-vascular systems will be discussed below (1.4).

One of the objectives of this thesis is to determine whether a relationship can be demonstrated between any of the above symptoms and oestrogen deprivation, as produced by castration.

1.4 METABOLIC EFFECTS OF THE CLIMACTERIC

In view of the fact that the menopause occurs at a time of life when there is a rising incidence of degenerative disease, it is not surprising that a causal relationship between some of these disorders and climacteric metabolic effects should have been postulated. Once again, proof of a direct relationship is lacking between oestrogen deprivation and development of any particular metabolic derangement or degenerative process. In some instances indirect evidence has been produced.

The metabolic effects of the climacteric will be described and discussed in relation to the development of specific degenerative or pathological processes.

1.4.1 HORMONE CHANGES

The climacteric as a phase characterized by important endocrinal changes associated with progressive loss of ovarian function is reviewed by Riley (1964). A number of theories have been adduced relating endocrine function to the symptomatology of the menopause. One of the most widely accepted theories (Albright, 1936) postulated that the symptoms are caused directly by the overproduction of human pituitary gonadotrophins (HPG) while another, that of 'oestrogen lack' (Heller et al, 1944b) proposes that the clinical features arise from a decrease in the levels of circulating oestrogens. There is a lack of information regarding hormone excretion patterns in subjects at or about the menopause and in the absence of such data an authoritative opinion cannot be given about the validity of the hypotheses mentioned above (Papanicolaou et al, 1969a).

It is generally accepted that the excretion of oestrogens in urine decreases with increasing postmenopausal age and decreasing ovarian activity. According to Brown and Matthew (1962) the values for total urinary oestrogen seldom exceed 10 μ g / 24 hours in postmenopausal women, a finding which is characteris-

tically associated with lack of endometrial stimulation due to absence of ovarian function. It is noteworthy that values as low as these may be encountered temporarily during the early proliferative phase of the menstrual cycle. Carcatzoulis (1963) reported that postmenopausal women always excrete oestrogen at a constant rate and the quantity is about one third of that excreted in normal menstruating women. Grönroos (1965) found no correlation between the total urinary oestrogen excretion and postmenopausal age. Furuhjelm (1966) concluded that the excretion of oestrogens in urine decreases with increasing postmenopausal age. It is generally agreed that the main source of oestrogen in postmenopausal subjects is the adrenal cortex (Loraine and Bell, 1966, 1968). Procope (1968), however, demonstrated two groups of postmenopausal women differentiated by ovarian histology, of which ovarian oestrogens were demonstrated in one group.

Loss of ovarian function is followed by a marked increase in the excretion of pituitary gonadotrophins. Papanicolaou et al (1969a, 1969b) have suggested that in late reproductive life the reciprocal relationship believed to exist between pituitary gonadotrophic function and ovarian oestrogen secretion is no longer operative and reported that an elevation in luteinizing hormone (LH) output to postmenopausal levels may precede the clinical menopause. The increase in HPG is predominantly due to an increased secretion of follicle stimulating hormone (FSH) although the levels of both FSH and LH are raised (Albert et al, 1961).

It is not yet clear whether in perimenopausal women the ovaries are under maximum stimulation by the pituitary or whether they are still capable of responding to stimulation with gonadotrophic hormones (Papanicolaou et al, 1969a),

although Poliak et al (1969) are of the opinion that the latter is possible.

Many clinical and experimental observations have suggested the existence of functional relationships between the adrenal cortex and the ovary (Jones, 1955; Turner, 1955). The excretion of total 17-hydroxy-corticosteroids, like that of total 17-ketosteroids, shows a steady decrease throughout the climacteric and postmenopausal years (Borth et al, 1957). Furthermore, there are indications that accelerated ageing of collagen may be attributed to increased ACTH formation (Verzár, 1968). It may thus be theorized that the pathway of decreasing ovarian function, decreasing adrenocortical function and resultant increased pituitary activity may be one of the factors in the aetiology of ageing human tissues.

1.4.2 CHOLESTEROL, CORONARY HEART DISEASE AND OESTROGENS

The endocrinological control of cholesterol metabolism is a complex subject. The interest of the present discussion is limited to the effect of endogenous and exogenous oestrogens on the serum cholesterol level and to demonstrating the reliability of these levels for the prediction of risk of developing atheromatosis and coronary artery thrombosis.

Some investigators have noted that the premenopausal female, compared with the human male, is protected against coronary accidents (Barr, 1953; Marmorston et al, 1962; Stamler et al, 1963; Schlesinger and Zoll, 1941). This sex difference is alleged to be due to oestrogens. The evidence will be examined.

The circulating cholesterol is largely synthesized in the liver and discharged into plasma bound to lipoproteins. The plasma cholesterol pool reflects the net result of a series of reactions which are donating sterol to the pool whilst others are removing sterol (Boyd, 1963).

Among Western populations and the prosperous moiety of non-White populations serum cholesterol and other blood lipids rise with age (Keys, 1963; National Centre for Health Statistics, 1966). Serum cholesterol is thought to increase from about 180 mg/100 ml in the 20s to about 220 in the 50s (Wyndham, 1969). The levels vary, however, in different population groups (Gram and Leverton, 1949; Walker, A.R.P., 1968; Wessel et al, 1963). For example, Barr (1953) recorded mean levels of 197 in normal women aged 18-35 and 252 in normal women aged 45-65. Oliver and Boyd (1959) reported mean levels of 217 for women aged 40-49, 240 for the groups 50-54 and 259 for the 55-59 year olds. The risks of coronary thrombosis are said to be seriously increased if serum cholesterol levels exceed 250 (Kannel et al, 1961; Wyndham, 1969).

Cholesterol is a precursor of oestrogenic hormones (Gual et al, 1962; Ryan and Smith, 1961). Endogenous oestrogens appear to influence cholesterol metabolism in at least two ways. There is an effect on the biosynthetic mechanism and also an influence on the rate of degradation or excretion (Boyd, 1963). Thus these oestrogens appear to exert an effect on human plasma cholesterol and lipid - lipoprotein levels (Barr, 1953; Katz and Stamler, 1953; Katz et al, 1958; Marmorston et al, 1962). In the human female oophorectomy produces an increase in the plasma cholesterol concentration of about 15% to 30% of the preoperative value (Oliver and Boyd, 1959), and spontaneous premature menopause may also result in elevation of these levels later in life (Sznajderman and Oliver, 1963).

Atheromatosis is a metabolic disease that is influenced by many factors (Bleyland and Wegener, 1969; Chapman and Massey, 1964; Drill and Riegel, 1958; Doyle et al, 1964; Katz et al, 1958; Kurland and Freedberg, 1960). The development of atheromatous lesions does not lend itself to precise measurement in the living. However, the extent and severity of coronary atheromatosis determines the risk of ischaemic heart disease in a given population (McGill et al, 1963). This presentation is concerned with only one phase of atheromatosis, namely, its relation to ovarian function and plasma cholesterol.

Coronary risk factors are those abnormalities demonstrable in persons free of coronary heart disease (CHD) and known to be associated with significantly increased risk of developing the disease in subsequent years. Numerous prospective studies on Western populations have indicated a close correlation between the moiety with elevated cholesterol levels and their proneness to ischaemic heart disease (Epstein, 1965; Katz et al, 1958; Kannel et al, 1961, 1964; Morris et al, 1966; Stamler, 1964; Stamler et al, 1966). Population groups with hypercholesterolaemia

experience four times as many heart attacks as those with low serum cholesterol levels (Stamler, 1964). This strong relationship is well shown in the Framingham study (Kannel et al, 1961, 1964; Thomas et al, 1966) and in the Los Angeles heart study (Chapman and Massey, 1964). Katz and Stamler (1953) have demonstrated experimentally a similar relationship. They noted that feeding cholesterol to cockerels produced coronary atherosclerosis.

In Western populations a similarity exists in the proportions of the leading causes of death (Mortality Statistics, 1967). Thus CHD accounts for about a third, cancer a quarter or less and 'strokes' 10% to 15% of total mortality. An important question, therefore, in improving mortality statistics is whether long-term reduction of hypercholesterolaemia will in turn reduce the incidence of atheromatosis and subsequent ischaemic heart disease. In this direction it is important to determine factors responsible for increasing and for decreasing plasma cholesterol levels (Walker, A.R.P., 1968).

Considerable evidence, experimental and clinical, is available indicating that endogenous ovarian oestrogen secretion plays a key role in protecting women against clinical atheromatous (atherosclerotic) coronary heart disease. This evidence is well reviewed by Berkson et al (1964) and key references only will be discussed below.

Numerous studies have been done on the effects of castration or premature menopause on the occurrence of both clinical heart disease and autopsy evidence of atherosclerosis in women (Higano and Cohen, 1963; Novak and Williams, 1960; Oliver and Boyd, 1959; Robinson et al, 1959; Wuest et al, 1953). Wuest et al (1953) compared the degree of sclerosis in hearts of bilaterally oophorectomized women with hearts of men and women of comparable ages. They found that the degree of coronary

sclerosis in the oophorectomized woman was greater than in control women but less than in control men. Oliver and Boyd (1959) reported that bilateral oophorectomy was followed by the premature development of clinical coronary artery disease; similar findings were found by Robinson et al (1959). Higano and Cohen (1963) reported the risk to be fourfold. Novak and Williams (1960) stated that the data of Robinson et al (1954) and Oliver and Boyd (1959), being clinical in nature, could not be as objective and precise as data obtained at autopsy. Regardless of initial age at operation or years intervening before death, they could find no significant differences in the incidence of atheroslerosis in statistically comparable groups of castrated and control patients at autopsy. A follow up study (Williams and Novak, 1963) suggested that operation before the age of 40 might be of significance. Parrish et al (1967) suspected that an important factor overlooked by previous writers was the time interval between castration and the 'expected date' of the normal menopause. From a study of autopsy records they found that castrated patients did have an excess of coronary atherosclerosis and myocardial infarcts, but this was directly related to the time interval from castration to 'expected menopause' and the time interval from castration to death. No excessive coronary atherosclerosis was found in women castrated after the age of 41 in contrast to those castrated when younger; excessive coronary atherosclerosis became apparent about 14 years after castration. They concluded that women castrated before the age of 40 who were expected to survive more than 14 years were at high risk of developing coronary heart disease. Finally, in a 6 - 20 year follow up study of 35 women who had undergone spontaneous premature menopause, Sznajderman and Oliver (1963) further supported the view that cessation of ovarian activity, whether premature or at the time of a normal menopause

leads to an increase in the incidence of ischaemic-heart disease and in serum-lipid levels in later life.

Thus the evidence as to the protective effect of the functioning ovary is convincing. Unfortunately, as yet the supporting evidence for the next logical step in this argument is not as sound, namely the effect of replacement exogenous oestrogen on the female cardiovascular system.

A wide range of oestrogenic substances have been studied for their effects on cholesterol metabolism (Drill and Riegel, 1958; Nestel et al, 1965). Oestradiol, oestradiol benzoate and diethylstilboestrol are more potent than oestrone as regards both lipid effects and oestrogenic activity. Ethinyl-oestradiol has less effect on lipids and is more potent as an oestrogen than oestrone (Drill and Riegel, 1958; Rubin et al, 1951). The table below is a summary from Boyd (1963) of a few of the oestrogens studied for their effect on cholesterol metabolism.

TRIVIAL NAME	APPROXIMATE OESTROGENIC DOSE	APPROXIMATE DOSE AFFECTING PLASMA CHOLESTEROL CONCENTRATION
	µg	µg
oestradiol-17β	200 - 500	25,000
ethinyl oestradiol	10 - 50	100 - 200
oestrone	1000 - 5000	50,000
oestriol	100 - 500	100,000
stilboestrol	100 - 1000	40,000

(after Boyd, 1963)

Thus, in the human, the administration of oestrogenic substances at certain dosages produces a depression in the plasma cholesterol concentration and the dosage necessary to produce this effect on the plasma cholesterol level is in excess of the threshold oestrogenic dose (Boyd, 1963). However, there is

insufficient data at this stage to allow more than speculation on the possible mode of action of the oestrogens in cholesterol metabolism.

Experimentally, Pick et al (1952) have shown that administration of oestrogens inhibits coronary atherogenesis in cockerels fed a cholesterol supplemented diet. There are no comparable studies in man. Oliver and Boyd (1961) administered oestrogen to myocardial infarction survivors. They found that, although significant reduction of the serum-cholesterol was evident throughout the five years of treatment, the continued reduction of serum lipids did not improve prognosis once myocardial infarction had occurred. Similar findings were reported by Marmorston et al (1962). Other studies have shown the effects of oestrogens on serum lipid patterns in postmenopausal women (Robinson et al, 1960; Barr et al, 1953). In most cases 1.25 mg of mixed conjugated equine oestrogens daily was adequate to produce a fall in serum cholesterol.

Numerous workers have attempted to prove that exogenous oestrogen therapy decreases the incidence of coronary heart disease, but there is no conclusive proof to date (Oliver and Boyd, 1961; Marmorston et al, 1962; Spritz, 1968; Stamler et al, 1963).

Despite the above lack of definite evidence there is a growing tendency towards the empirical use of long term exogenous oestrogen therapy in the postmenopausal woman to prevent ischaemic heart disease (Davis et al, 1961; Davis, 1964; McBride, 1967; McEwen, 1965; Wilson and Wilson, 1963; Wilson et al, 1963).

The discussion presented illustrates that there are still many aspects of this complex inter-relationship requiring further elucidation or confirmation. The present study was planned to evaluate just two of these aspects, namely the effect of oophorectomy on plasma cholesterol levels and whether exogenous

oestrogen administration to identical and statistically comparable groups of patients can cause a lowering of these values.

1.4.3. CALCIUM AND PHOSPHORUS METABOLISM AND OSTEOPOROSIS

The decreasing ovarian function of the climacteric is said to be a factor leading to the development of osteoporosis. Postmenopausal osteoporosis has been the subject of several reviews (Bassan et al, 1963; Frost, 1961, 1966; Henneman, 1964) and the present discussion is limited to defining the problem in terms of the relationship between oestrogen deficiency or oestrogen therapy, calcium and phosphorus metabolism and osteoporosis.

It was not until 1941 that Albright et al (1941) described osteoporosis as a clinical entity. Osteoporosis is a common clinical disorder which may cause backache or fracture in the elderly. It is characterised by a reduced amount of bony tissue per unit volume of bone in the affected part or parts of the skeleton. Unlike osteomalacia, there are no known specific biochemical abnormalities in osteoporosis and most authors agree that osteoporotic bone, while reduced in quantity, is essentially normal in chemical composition (Nordin et al, 1966).

The investigation of osteoporosis has been handicapped by the lack of satisfactory objective radiodiagnostic criteria. The Medical Research Council's Mineral Metabolism Unit has developed two quantitative measures (Brit. Med. J. 1969). The first method is X-ray densitometry and the second isotope densitometry. Lanzl and Strandjord (1965) have also reported on a radio-isotopic device for measuring bone mineral. Unfortunately none of these techniques could be made available in Cape Town at the initiation of the present study.

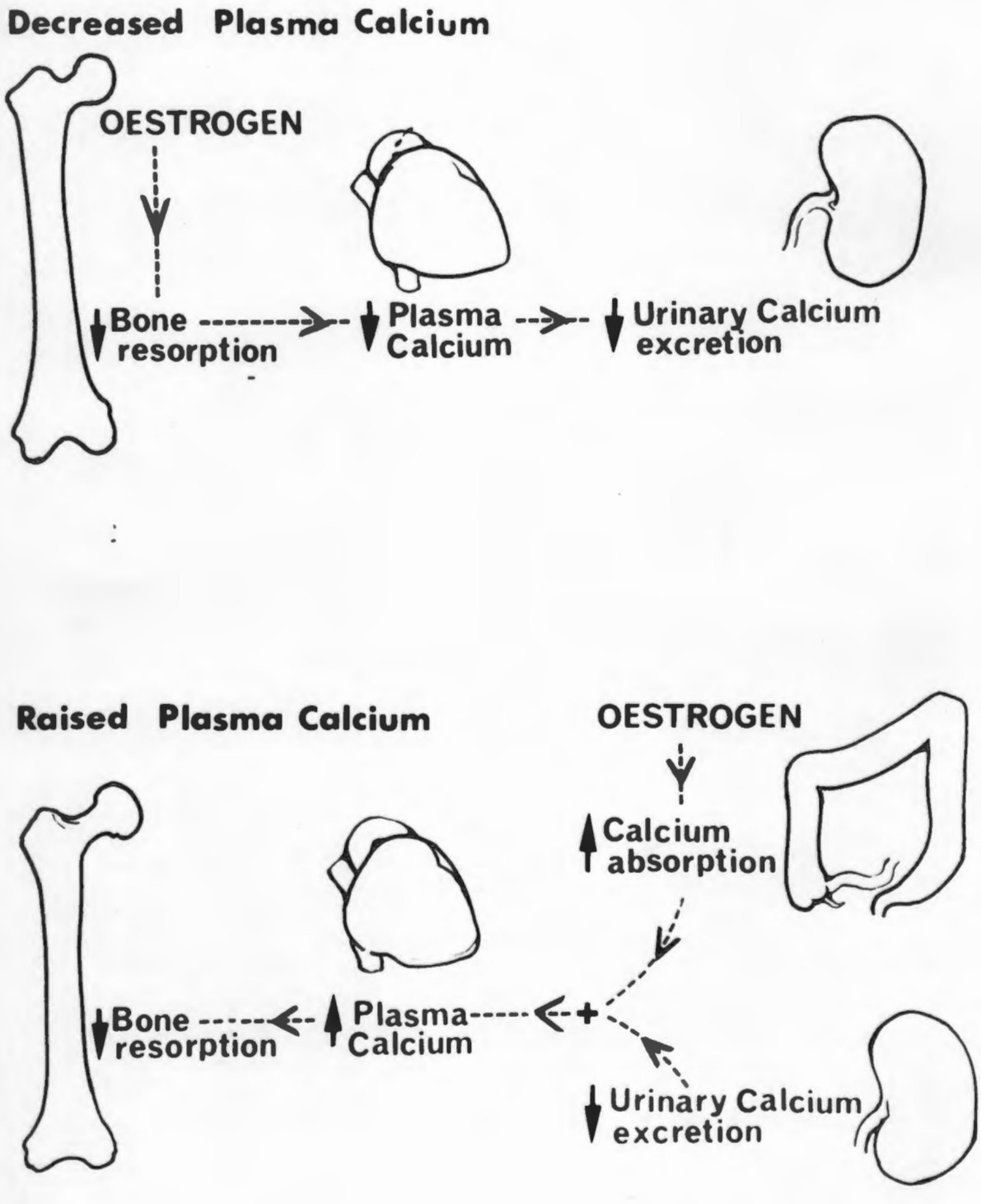
The pathogenesis of osteoporosis is obscure. The

osteoporotic process commences or accelerates in women soon after the natural or induced menopause (Albright et al, 1941; Meema et al, 1965; Meema, 1963; Nordin et al, 1966; Saville, 1967). For this reason Albright et al (1941, 1948) attributed the condition to gonadal insufficiency resulting in a reduced rate of synthesis of bone collagen (matrix). This theory has not been substantiated by most isotope kinetic studies (Heaney and Whedon, 1958; Nordin, 1959) and tends to be refuted by histological evidence suggesting bone resorption and normal bone formation (Frost, 1961, 1966). This recent work (reviewed by Nordin et al, 1964) demonstrating increased bone resorption possibly related to calcium deficiency (Harrison et al, 1961) rather than decreased bone formation as the likely cause of osteoporosis has not only cast doubt on the collagen theory but on the role of the gonadal hormones as well (Lafferty et al,1964). Although doubt has been accentuated by the absence of convincing evidence that osteoporosis responds to treatment with gonadal hormones, it does appear that osteoporosis is related to endogenous oestrogen deficiency (Nordin et al, 1966), the evidence being circumstantial and not all workers in agreement (Ruikku et al, 1968; Fourman and Royer, 1968). It seems that, although the osteoporosis of postmenopausal women may ultimately be due to hormonal deficiency, its immediate cause must lie in the regulation of calcium absorption or excretion (Bronner et al, 1963; Nordin et al, 1966; Jasani et al, 1965; Young and Nordin, 1967).

Factors as described above have focused attention on the menopause and therefore on the effects of the oestrogenic hormones on calcium metabolism. Jasani et al (1965) considered that careful measurement of plasma calcium might be one way of distinguishing whether the negative calcium balance of osteoporosis is secondary to a primary rise in bone resorption,

FIGURE I

THEORETICAL EFFECT of OESTROGEN on PLASMA CALCIUM



or the rise in bone resorption is the result of an external negative calcium balance. They theorised that 'if the loss of oestrogenic activity has a direct effect upon bone resorption and increases the release of calcium into the plasma', it will tend to raise the serum calcium and so also the urinary calcium. If, on the other hand, oestrogens tend to increase either the renal tubular reabsorption of calcium or the absorption of calcium from the small intestine (or both) then deficiency of oestrogenic hormones (unless associated with an increased calcium intake) would tend to lower the plasma calcium and consequently increase the rate of bone resorption in order to maintain the plasma level. Thus the difference between these two processes is in their effect on plasma calcium (see diagramatic representation, Figure I). According to one hypothesis, oestrogens would tend to lower urinary calcium and so to raise the plasma level and reduce bone resorption; according to the other, oestrogens would tend to lower plasma calcium and so to lower urinary calcium and improve calcium balance'. Their preliminary results suggested that oestrogens do not raise the plasma calcium and that the menopause (oestrogen deficiency) is associated with a slight rise in plasma and urinary calcium. They therefore suggested that oestrogenic hormones have an action on the blood/bone equilibrium antagonistic to that of parathyroid hormone and that a reduction of oestrogenic activity leads to a marginal elevation of plasma calcium and so to hypercalciuria and to negative calcium balance. They have suggested, moreover, that parathyroid hormone can break down bone more easily when oestrogens are not present. The degree of osteoporosis which this produces must depend upon the length of time which elapses before the subject re-adapts to the new situation. A further report (Young and Nordin, 1967), showed a significant rise in plasma and urinary calcium and phosphorus values after the

menopause. In the premenopausal women, fasting plasma calcium was 9.32 and the phosphorus 3.31 mg/100 ml. After the natural menopause the corresponding values were 9.62 and 3.52. After the artificial menopause the corresponding values were 9.81 and 3.62. The rise of plasma and urinary calcium was most apparent during the first years after the cessation of menstrual periods. They interpreted these changes to mean that the menopause is followed by a rise in bone resorption which leads to a rise in plasma and urinary calcium and phosphorus and Szymendera and Madajewicz (1967 have reported similar findings.

Although the above biochemical changes can be reversed by oestrogenic hormones (Young et al, 1968) the evidence as to improvement of osteoporosis following such therapy is poor. Lafferty et al (1964) found that oestrogens primarily reduce bone resorption and do not stimulate bone formation. In fact, after prolonged administration a secondary decline in bone formation occurred. Thus, despite publications suggesting prophylactic oestrogen therapy and reports on relief of symptoms, there is little evidence of increased bone formation by densitometry, X-ray or histological studies (Bassan et al, 1963; Davis et al, 1966; Fourman and Royer, 1968; Hernberg, 1960; Lafferty et al, 1964; Meema and Meema, 1968; Wallach and Henneman, 1959).

The present study was planned to incorporate an investigation into plasma calcium, phosphorus and alkaline phosphatase levels. Thus there was no attempt to demonstrate the development of frank osteoporosis; rather an attempt was made to prove one step in the pathogenesis of the disease.

Plasma alkaline phosphatase levels were performed for completeness' sake. There are two important causes for raised serum alkaline phosphatase activity; those diseases of

bone in which there is increased osteoblastic activity and diseases of the liver, especially where there is obstruction to the outlow of bile (Brit. Med. J., 1968). These levels, therefore, have an established place in the investigation of suspected diseases of bone.

1.4.4. VAGINAL SMEAR

Since the early work of Stockard and Papanicolaou (1917) numerous workers have confirmed the relationship between oestrogen metabolism and the cells of the vaginal epithelium.

The literature on postmenopausal vaginal smear has been extensively and thoroughly reviewed by Grönroos (1965). The use and methodology of exfoliative cytology for the evaluation of endocrinological conditions has been reviewed by Liu (1968), Rakoff (1961) and Wied (1968).

The proportion of atrophic smears has been found to increase with the postmenopausal years (Masukawa, 1960) although an oestrogen-like effect may be observed in many postmenopausal women (Stone et al, 1967) and there is difficulty in overcoming differences attributable to age disparity (Stern et al, 1966).

The vaginal smear is a bioassay. Relatively few correlative studies have been published on the urinary oestrogen output and the oestrogen effect in vaginal smear. Grönroos (1965) found a positive correlation between karyopyrotic index (KI) and urinary output of oestrone, oestradiol and oestriol in healthy postmenopausal patients. Procopé (1968) confirmed this correlation.

The hormonal evaluations are usually expressed by means of indices depending upon the variations between the accepted three major cell types (superficial, intermediate and parabasal cells). The maturation index (Frost, 1958), for example, reflects the percentage of superficial, intermediate and parabasal cells

present and has the advantage of easy transfer to the computer for analysis (Stone et al, 1967).

Despite the validity of the vaginal smear as a bioassay of the oestrogenic status of the postmenopausal female there is doubt as to the use of the vaginal smear as an assessment of the need for or response to exogenous oestrogen therapy (Kaufman, 1967).

A further object of the present study was to determine the effect of the surgical menopause (castration) and subsequent exogenous oestrogen therapy to the same group of patients on the vaginal smear. It was hoped to correlate the vaginal smear with urinary oestrogen levels. Unfortunately it was not financially possible to establish a technique to measure total or fractionated urinary oestrogens in the Department of Gynaecology.

1.5 OOPHORECTOMY - INDICATIONS AND INCIDENCE

The surgical removal of normal ovaries at the time of routine abdominal hysterectomy performed for benign conditions is a controversial subject in modern gynaecology.

The reasons advanced for removal of ovaries are as follows :

1. Prevention of subsequent development of cancer in the retained ovaries.
2. The theory that retained ovaries have diminished or absent function.
3. High incidence of repeat laparotomy for ovarian pathology (Grogan and Duncan, 1955).

The ovaries are frequently retained in order to :

1. Prevent development of postoperative menopausal symptoms (discussed under section 1.3).
2. Prevent regressive effects related to oestrogen withdrawal (discussed under sections 1.3 and 1.4).
3. Prevent development of osteoporosis (discussed under 1.4.3).
4. Prevent development of ischaemic heart disease (discussed under section 1.4.2.).
5. Psychological reasons.

The possible clinical and metabolic effects of oophorectomy are well reviewed by Murless (1964) and are discussed under sections 1.3 and 1.4. The indications advanced for removal of normal ovaries are discussed below.

The risk of subsequent development of carcinoma in retained ovaries following hysterectomy is generally considered to be small. Schabort (1960), reviewing reasons for retaining ovaries, reported the incidence to be approximately 2 in 5,000 hysterectomies. This figure is similar to that of 1 - 3,000 to 5,000 hysterectomies quoted by Jeffcoate (1967). However, Bloom (1962) found that 15 (10.6%) of 141 cases of primary

adenocarcinoma of the ovary from the Johannesburg General Hospital had previously had a hysterectomy or other pelvic operation performed. In 5 of these cases (3.5%) the ovaries had been conserved when the patient was over 50. Counseller (1955) reported on 1,500 cases of proved carcinoma of the ovary of whom 67 (4.5%) had undergone hysterectomy for a benign condition. While 33% of these 67 patients were in the group less than 40 years of age, only 4.5% of the ovarian carcinoma occurred in that age group. They suggested that oophorectomy should therefore be performed only on patients over the age of 40. Nevertheless, until the results of further large surveys are analysed it is not possible to define the absolute risk of subsequent development of carcinoma.

It has been said that following hysterectomy the ovaries soon become functionless. There is, however, evidence to show that the ovaries may continue to function for several years after hysterectomy (Bancroft-Livingston, 1954; Heller et al, 1941; Richards, 1951; Whitelaw, 1958). There is furthermore no indication that hysterectomy hastens the onset of the menopause (Whitelaw, 1958) and Randall et al (1957) have even suggested that the ovaries may produce oestrogen after the menopause. Additional evidence to show that conserved ovaries function completely normally up to the expected time of the menopause was reported subsequent to the completion of this thesis (Beavis et al, 1969).

The incidence of surgical removal of ovaries at hysterectomy varies according to current fashion in different gynaecological units. Randall and Paloucek (1968) reported that no generally accepted criteria for ovarian preservation or indication for ovarian removal had been observed in one geographic area during the 25 year period reviewed. It is of interest that in the annual Clinical Reports of the Division of Gynaecology,

University of Cape Town, no statistics relating to removal or preservation of ovaries are included. Hence an analysis in this respect in Cape Town is not possible.

There is therefore good evidence, as described in the aforegoing discussion, that there are advantages to the conservation of normal functioning ovaries at the time of removal of the uterus. An unanswered question,nevertheless, is whether exogenous oestrogen therapy following bilateral oophorectomy has the same or similar metabolic effects as retained functioning ovaries. The present thesis attempts to evaluate part of this question.

1.6 OESTROGEN REPLACEMENT THERAPY

The general management of the climacteric has been the subject of many reviews (Greenblatt, 1963; Jeffcoate, 1960, 1967; Rogers, 1956b). There is also a large literature concerning the various preparations of oestrogens and the several routes of administration (Brown et al, 1951; Cohen, 1968; Haskins et al, 1962; Jeffcoate, 1960; Kupperman et al, 1953; Kupperman and Blatt, 1959; Swyer, 1959). Many reports testify to the use of various oestrogens for the relief of 'postmenopausal symptoms' (cf Section 1.3) (Dapunt, 1967; Greenblatt et al, 1962; Greenblatt, 1965; Hankin, 1967; Sands, 1967; Wilson et al, 1966). Some authors suggest a general tonic effect with exogenous oestrogen therapy (Davis, 1967; Greenhill, 1967; Wilson and Wilson, 1963) and a preliminary report states that such therapy may prevent psychological deterioration in the older woman (Kantor et al, 1968). Despite this there is a remarkable lack of comparative clinical trials in gynaecology, and the choice of an oestrogen has still to be based on relatively uncontrolled collective experience (Drug and Therapeutics Bulletin, 1968). For this reason part of the present study entailed a controlled comparative evaluation between two forms of oestrogen and a placebo.

There has been a recent tendency to regard the climacteric as a hormone (oestrogen) deficiency syndroms. Wilson and Wilson (1963) have led this school of thought and make a plea for 'the maintenance of adequate oestrogen from puberty to the grave'. In 1963 Wilson et al reported on 'specific procedures for the elimination of the menopause'. Support has come from other writers (Bedford, 1967; Davis, 1964, 1965, 1967; Greenblatt, 1963; Kushima et al, 1961; McEwen, 1965; McBride, 1967). The arguments presented by these authors for such therapy are incomplete and in many instances have no basis in fact. The essential fallacy is

that there is little evidence to show that exogenous oestrogen therapy has the same effect as that of the endogenous ovarian oestrogens (discussed in section 1.4). There is thus a strong school of thought in disagreement with such empirical therapy (Greenhill, 1967; Novak, 1967; Taylor, 1968).

Furthermore, long-term exogenous oestrogen therapy may not be without risk. There is an increasing literature relating oestrogen therapy with vascular thrombosis and embolism when used for suppression of lactation or in the contraceptive pill (Daniel et al, 1967; Jeffcoate et al, 1968; Poller and Thomson, 1966; Poller et al, 1969; Swyer, 1966; Vessey and Doll, 1968, 1969).

There is no direct evidence to suggest a carcinogenic effect of oestrogen. Cases have been reported in which prolonged administration has coincided with the development of uterine or breast carcinoma and this aspect is fully reviewed by Rogers (1956b) and Larson (1954). It is possible to state that in this respect the concern over the use of oestrogens does not seem justified by a review of the available evidence.

PART 2

OBJECTIVES OF THE PRESENT STUDY

2. OBJECTIVES OF THE PRESENT STUDY

The present investigation was undertaken in order to clarify the clinical and metabolic effects of the menopause following bilateral oophorectomy and to evaluate the role of replacement exogenous oestrogen therapy. To this end the following studies were carried out :

1. A study of the clinical effects directly related to ovarian oestrogen withdrawal following bilateral oophorectomy.
2. To determine, should any clinical effects be found, whether such effects could be reversed by exogenous oestrogen replacement therapy.
3. A study of the changes in vaginal smear following bilateral oophorectomy and subsequent oestrogen therapy.
4. A study of the changes in plasma cholesterol in patients undergoing bilateral oophorectomy and the effect of administration of exogenous oestrogen in these patients.
5. A study of the effect of bilateral oophorectomy on plasma calcium, phosphorus and alkaline phosphatase values and the result of subsequent exogenous oestrogen therapy to the same group of patients.
6. A consideration of the clinical and metabolic effects of bilateral oophorectomy with particular reference to the place of this procedure in gynaecology at the time of routine abdominal hysterectomy for benign conditions; and to consider further the role of exogenous oestrogen replacement therapy in all females after the menopause.
7. A controlled comparative evaluation of two different forms of commercially available oestrogen and a placebo.

PART 3

MATERIALS AND METHODS

3.1 MATERIAL

The principal aims of the study required a detailed clinical history and examination and the collection of material from varying groups of women whose samples would reflect an average physiological status for the group in question. The acquisition of this comprehensive material on sufficient patients and on repeated occasions over a period of one year for each patient posed a difficult organizational problem. Accordingly, a special 'Menopause Clinic' was established in the Outpatient Department of the Groote Schuur Hospital, Cape Town. This was conducted at a regular time each week for two years. The study comprised inpatients gathered for follow-up and outpatients who were recalled following a survey of the records of the Department of Gynaecology. Many persons and samples had subsequently to be excluded owing to incomplete follow up or attendance or failure to fulfil the criteria listed below. As far as possible, however, patients, once entered into the study, were persuaded from defaulting.

The number of patients who were studied in the course of the investigations described in this thesis is such as to render it unpractical to include individual case reports. However, patients were gathered in several groups according to the following criteria :

Points of similarity

1. Females aged 45 to 55
2. Absence of gynaecological or other diagnosed malignancy
3. Absence of vaginal treatment in the preceding 2 months prior to the first visit
4. They had not received hormonal, vitamin or digitalis therapy in the preceding 6 months
5. There were no symptoms of general disease
6. The patients were all Caucasians (white) of similar socio-economic status

Variable features

Group 1 : Premenopausal - intact uterus and ovaries

Group 2 : Premenopausal - investigation commenced immediately post oophorectomy

Group 3 : Premenopausal - investigation commenced 6 months post oophorectomy

Group 4 : Premenopausal - investigation commenced 2 years post oophorectomy

Group 5 : Postmenopausal - investigation commenced immediately post oophorectomy

Group 6 : Control - 6 months post hysterectomy with conserved ovaries

Group 7 : Control - 2 years post hysterectomy with conserved ovaries

The ultimate number of patients completely studied was 85, divided into two major sub-groups. The first group comprised 50 patients (groups 2, 3, 4 and 5) who formed the detailed material for study and were thus investigated and treated on 6 occasions each. The second group of 35 patients (groups 1, 6 and 7) was seen and investigated on one visit for control purposes only

The 85 patients were divided between the minor sub-groups as follows :

GROUP NO.	DESCRIPTION	NO. OF PATIENTS	
1	Normal premenopausal	9	
2	Premenopausal investigated immediately post oophorectomy	14	50
3	Premenopausal investigated 6 months post oophorectomy	13	
4	Premenopausal investigated 2 years post oophorectomy	18	
5	Postmenopausal investigated immediately post oophorectomy	5	
6	6 months post hysterectomy with conserved ovaries	8	
7	2 years post hysterectomy with conserved ovaries	18	

It will be observed that all patients to receive treatment (Groups 2, 3, 4 and 5) had undergone oophorectomy. The post-oophorectomy groups were selected for detailed study because the climacteric cannot be accurately timed. Hence it is virtually impossible to collect a study group of human material at an accurately timed menopause other than in this way. Thus evaluation of oophorectomized patients allows of precise analysis of features related to ovarian oestrogen withdrawal.

At first inspection the number of patients in each sub-group appears small. It was not possible to enlarge the size of the groups in the time available as the patients investigated comprise the total clinical material obeying the criteria for selection and passing through Groote Schuur Hospital at the time of this investigation.

Allowance has therefore been made for this in the statistical evaluation.

The figures shown above excluded investigation of the several patients who failed to attend for the full scheme of treatment. In all instances except one the reason related to departure from Cape Town. One patient, however, died between the 5th and last visit. The cause of death was described at post-mortem as being due to :

> "Faecal peritonitis, perforation at the caecal-colonic junction and multiple peritoneal and pelvic adhesions".

The data obtained for this patient was included in the final analysis. Data for the other patients in Groups 2, 3, 4 and 5 attending on less than 5 of the 6 required visits was excluded on the grounds of being incomplete.

3.2. METHODS

3.2.1 GENERAL

All patients studied had the same history, examination and investigations performed at each visit. Accordingly, it was possible to establish a set protocol or trial form. In view of the anticipated amount of data that was to be collected, this form was drafted at the outset of the trial to allow for computer analysis. Slight alterations that occurred during the study necessitated revision of the form.

The trial form lists symptoms, signs and investigations that were assessed. The mode of assessment or translation to a numerical measurement is explained by the code sheet (Trial form and explanatory code - see Appendix A).

After completion of the study all data was transferred on to IBM 80-line computer punch cards for subsequent analysis and statistical evaluation through an IBM 1130 computer.

3.2.2. CLINICAL EVALUATION

Details of symptomatology and changes in clinical signs were determined according to accepted clinical criteria.

Symptoms, for the purpose of comparison,were graded as being absent, slight, moderate or severe. The development of the breasts was assessed as being of the firm, rounded type of reproductive years or else of atrophic, flabby postmenopausal type.

A full-face colour photograph was taken of all patients in groups 2,3,4 and 5 at each visit to the clinic. The purpose of this procedure was to record any change in facial features, wrinkling of skin, scalp hair-line configuration or facial hair. The inclusion in this thesis of prints of the hundreds of photographs taken during the course of this study was impractical. Thus, reference to the results of this investigation will be incorporated in Part 5.1 only.

Further details of actual symptoms and signs measured are reflected and listed in the trial sheet and explanatory code (Appendix A).

It is necessary to comment on factors 74 (overall impression), 79 (patient's assessment) and 80 (husband's assessment). These were scores determined at each visit following the completion of history taking, clinical examination and collection of samples for investigation and applied to all patients receiving therapy (i.e. groups 2, 3, 4 and 5). The patient was asked how she felt generally and specifically how she felt in comparison to the previous visit. Factors taken into account were as follows :

1. Degree of general feeling of 'well-being' and interest in life
2. General ability to perform a full day's work, i.e. degree of fatigue in response to work
3. Whether side effects of therapy detracted from any possible beneficial effects

4. Ability to maintain satisfactory inter-personal relationships without excessive irritability, anxiety, impatience, etc.

The above factors were balanced into an overall impression and awarded a score as follows :

1 = marked improvement

2 = slight improvement

3 = unchanged

4 = slight deterioration

5 = marked deterioration

The husband's assessment was discontinued early in the study due to the high incidence of divorcees, widows and disinterested husbands.

It is conceded that an 'impression' is not a scientific measurement. Nevertheless, all patients were assessed by the author only and points awarded as far as is humanly possible without bias.

The value of this assessment is considered to be purely a measurement of euphoria or effect on the mental state of the patient, produced either as a drug response or the result of physician's care (placebo effect). No similar measurement appears to have been attempted previously. Nevertheless, despite the wide margin of error possible, this evaluation was considered to be both necessary and of potential value in testing claims for the 'tonic effect' of long-term oestrogen therapy, as discussed under section 1.6

3.2.3 METHODS FOR THE DETERMINATION OF BLOOD CHOLESTEROL, CALCIUM, PHOSPHORUS AND ALKALINE PHOSPHATASE VALUES

Cholesterol, calcium, inorganic phosphorus and alkaline phosphatase values were determined by standard methods in the Department of Chemical Pathology, University of Cape Town.

Cholesterol : The procedure followed in the Department of Chemical Pathology for the determination of cholesterol in serum is described by Pearson, Stern and McGarack (1953) and has been slightly modified by Duncan (1959). Results are presented in mg per 100 ml. Normal values by this method are 150 - 250 mg per 100 ml.

Calcium : Plasma calcium values were determined by high atomic absorption spectrophotometry. Results are presented in mg per 100 ml. Normal values for this laboratory are 8.5 to 10.6 mg per 100 ml.

Inorganic phosphorus : Phosphorus values were determined by the standard clinical method of Fiske and Subarrow (1929). Results are presented in mg per 100 ml, the normal values ranging between 2.8 and 4.5.

Alkaline phosphatase : Alkaline phosphatase was determined on the Auto-Analyser using phenolphthalene monophosphate as substrate with results corrected to Bodansky-Reinhart units. The normal value for this laboratory is less than 12 Bodansky-Reinhart units.

3.2.4 VAGINAL SMEAR

This aspect of the investigation was personally conducted in the Cytology Laboratory of the Department of Gynaecology with the assistance of one technician.

The smears were taken with a wooden spatula from the lateral fornix. Care was taken to avoid traumatisation of the epithelium. The inclusion of cells from the outer part of the genitals was prevented by using a speculum. The material obtained was spread gently, without rubbing, on a glass slide. The slide was immediately sprayed with an aerosol-type fixative consisting of normal propyl alcohol, polyethylene glycols 400 and

1500, trichloro-monofluro-methane (frion 11) and dichloro-difluro-methane (frion 12). The fixation time varied from one hour to two days.

Vaginal smears were stained by the method of Papanicolaou and Traut (1943).

There is a diversity of terms and definitions used for the cells that have desquamated from the squamous epithelium. These are reviewed by Grönroos (1965) and Wied (1968). The definitions in this study were as follows :

(i) Parabasal cells were determined on a basis of cellular size, the limits being a diameter of 15 - 30 μ, and shape, the cells demonstrating a round or oval circumference with no angulation.

(ii) Intermediate cells were identified as squamous cells presenting a definite differentiation of cytoplasm, a beginning retraction of the nuclear diameter, but without complete karyopknosis (International Academy of Gynaecological Cytology, 1958).

(iii) Superficial cells were distinguished from other cells above all by their pyknotic nucleus. In this study a pyknotic nucleus was defined as one which does not exceed 5.5 μ in diameter and appears black and structureless (de Neef, 1967; Grönroos, 1965; Hindman et al, 1962; Wachtel, 1964).

The counting of cells was performed as follows. Under a low magnification (8 x 10) and in a bright field a clear area was selected containing separate epithelial elements. A total of 500 cells was identified and counted in vertical and horizontal directions under a magnification of 8 x ocular and 45 x objective. The size of the pyknotic nucleus was measured at the same time with an ocular micrometer. Cells that were too degenerated or too clumped were not included.

The number of parabasal, intermediate and superficial cells were corrected to percentages and placed in that order e.g. 7 : 63 : 30. The Maturation Index (Frost, 1958) thus obtained allowed for easy transfer to the computer for statistical evaluation.

3.2.5 TREATMENT PROGRAMME

The 50 patients comprising groups 2, 3, 4 and 5 were each evaluated on 6 occasions over a period of one year. On each visit all investigations were repeated before treatment was prescribed or changed according to the following schedule :

VISIT NO.	DRUG ADMINISTERED AFTER INVESTIGATION COMPLETED	TIME LAPSED SINCE PREVIOUS VISIT
1	Oestradiol valerate 4 mg daily	-
2	Oestradiol valerate 4 mg daily	1 month
3	Oestradiol valerate 4 mg daily	2 months
4	Oestradiol valerate 4 mg daily	3 months
5	Placebo - Single blind	3 months
6	Conjugated equine oestrogen 5 mg daily	3 months

Thus the data obtained on each visit was a reflection of the following status :

VISIT NO.	DESCRIPTION
1	Control - variable time post oophorectomy
2	1 month continuous oestradiol valerate therapy
3	3 months continuous oestradiol valerate therapy
4	6 months continuous oestradiol valerate therapy
5	3 months continuous placebo therapy - single blind
6	3 months conjugated equine oestrogen therapy

No results for visit 2 (1 month continuous oestradiol valerate therapy) are presented in this thesis. This visit was essentially an early check to determine that patients were taking therapy as prescribed.

The substitution of placebo for oestradiol valerate was performed on a single blind basis. The tablets were identical in appearance and the patients were kept uninformed of the substitution. Unfortunately it was not possible to have an identical type tablet of conjugated equine oestrogen. Following the substitution at visit 5 the patients were informed that the hormone was the same but the processing of the tablet was of a different nature. In the majority of instances this was accepted without comment. In all instances the drugs were administered continuously with no cyclical variation.

The choice of oestrogens used was decided in view of their both being described as natural oestrogens (Manufacturers' information) :

Oestradiol valerate (Progynova, Schering) is a fatty acid ester of the naturally occurring follicular hormone oestradiol and, parenterally administered in animals, has an oestrogenic effect of similar potency to that of oestradiol. The valeric acid ester is excreted via the lungs. Orally administered, 60 mg of oestradiol valerate is sufficient to achieve full endometrial proliferation in a female castrate.

(Manufacturers' information - Schering A.G., Berlin)

Conjugated equine oestrogens (Premarin, Ayerst) are water soluble oestrogens derived from pregnant mares' urine. They comprise oestrone sulphate (over 70%), equilin sulphate, 3-monosulphate of oestradiol - 17a, 3-monosulphate of dihydroequilenin - 17a and other conjugated steroids. The proliferative dose is 80 mg (Manufacturers' information - Ayerst Laboratories, New York).

The dosage selected was related to the proliferative dose as follows :

Proliferative dose of Oestradiol valerate = 60 mg

Proliferative dose of Conjugated oestrogens = 80 mg

Hence by calculation a dose of 4 mg of oestradiol valerate was considered to be equivalent to 5 mg of conjugated equine oestrogens for the evaluation of other oestrogenic effects.

3.2.6 STATISTICAL ANALYSIS

All data was analysed through an IBM 1130 computer with the assistance of a computer programmer and statistician (see ACKNOWLEDGEMENTS).

The statistical tests applied were basically the student t distribution to pure measurements (e.g. weight, cholesterol, calcium, phosphorus and alkaline phosphatase) and the chi square distribution test to clinical features (e.g. hot flushes, headache, irritability, etc.)

In this study the differences or correlations are considered statistically almost significant if $p \leq 0.05$, significant if $p \leq 0.01$ and highly significant if $p \leq 0.001$.

For the purpose of completeness of presentation, comparisons between all study groups for statistical significance are listed under the individual headings of Part 4 - RESULTS. As all comparisons may not be of importance for any one parameter, only the relevant comparisons will be commented on in the accompanying text to each table.

3.2.7 NOTE ON DISCONTINUED INVESTIGATIONS

Two investigations were discontinued early in the course of this study :

1. Glucose tolerance test : This investigation was discontinued because most patients were loath to submit themselves to this procedure for as many as six times in one year. Analysis of the results that were obtained was of small value and such results have accordingly been excluded from this thesis.

2. Radiographs of lumbar spine : X-rays of the lumbar spine were taken at every patient's first visit and repeat radiographs were taken on several occasions in some instances. This procedure, however, was found to be valueless for the accurate assessment of osteoporosis and, as the emphasis in this study was on metabolic aspects, the procedure was discontinued.

PART 4

RESULTS

4.1 ANALYSIS OF SYMPTOMS

4.1.1 HOT FLUSHES

The overall incidence and degree of severity of hot flushes in all groups of patients studied is shown in Tables I and 2. The response of hot flushes in oophorectomized females to oestrogen and placebo therapy is outlined in Table 2. The statistical comparison between the groups of patients investigated and the overall incidence of hot flushes of all degrees of severity is tabulated in Table 3.

There is a low incidence of hot flushes in the normal premenopausal group (11.1%) and the groups of patients undergoing hysterectomy with conservation of ovaries (12.5% at 6 months and 16.7% at 2 years). These results also suggest that the incidence of hot flushes does not significantly increase for at least 2 years after such surgery, i.e. the conserved ovaries continue to function for at least 2 years.

Following oophorectomy there is a highly significant increase in the incidence of this symptom ($p < 0.001$). The incidence is increased 6 months after oophorectomy ($p < 0.01$), but does settle slightly at the 2 year mark ($p < 0.01$).

Comparison of the oophorectomized to the non-oophorectomized groups reveals highly significant differences ($p < 0.001$). Retention of ovaries decreases the likelihood of the development of hot flushes.

The response of hot flushes to oestrogen administration is striking ($p < 0.001$) with relief of the symptom in the majority of patients. This cure is not a placebo effect as single blind cross-over to placebo results in return of the symptoms in a high proportion of cases (66%) and this finding is highly significant ($p < 0.001$).

Both forms of oestrogen used in this study were effective ($p < 0.001$) and no statistical difference in effect between the two hormones could be demonstrated.

Thus the symptom of hot flushes has been shown to be oestrogen dependent - oophorectomy results in a striking increase in the incidence of hot flushes and oestrogen therapy significantly relieves this symptom.

TABLE 1

INCIDENCE AND SEVERITY OF HOT FLUSHES IN THE GROUPS OF PATIENTS INVESTIGATED

HOT FLUSHES	NORMAL		POST-OOPHORECTOMY								CONSERVED OVARIES			
	PREMENOPAUSAL		PREMENOPAUSAL						POSTMENOPAUSAL		POST-HYSTERECTOMY			
			Immediate		6 months		2 years		Immediate		6 months		2 years	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients studied	9	100	14	100	13	100	18	100	5	100	8	100	18	100
No. of hot flushes per 24 hours ABSENT	8	88.9	7	50.0	0	0	7	38.9	2	40.0	7	87.5	15	83.3
1 - 3	1	11.1	4	28.6	10	76.9	10	55.6	3	60.0	1	12.5	3	16.7
4 - 6	0	0	3	21.4	3	23.1	0	0	0	0	0	0	0	0
> 7	0	0	0	0	0	0	1	5.6	0	0	0	0	0	0

TABLE 2

THE INCIDENCE OF HOT FLUSHES IN OOPHORECTOMIZED FEMALES AND THE RESPONSE TO OESTROGEN AND PLACEBO THERAPY

HOT FLUSHES	CONTROL		3 MONTH OESTRADIOL VALERATE		6 MONTH OESTRADIOL VALERATE		3 MONTH PLACEBO		3 MONTH CONJUGATED OESTROGENS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100
Absent	16	32.0	47	94.0	45	90.0	17	34.0	42	85.7
1 - 3 per day	27	54.0	3	6.0	4	8.0	17	34.0	7	14.3
4 - 6 per day	6	12.0	0	0	1	2.0	12	24.0	0	0
> 7 per day	1	2.0	0	0	0	0	4	8.0	0	0

EXPLANATION OF GROUP NUMBERS DESCRIBED
IN TABLE OF STATISTICAL COMPARISON

GROUP NO.	DESCRIPTION OF GROUP OF PATIENTS
1	Normal premenopausal
2	Premenopausal, investigated immediately post-oophorectomy
3	Premenopausal, investigated 6 months post-oophorectomy
4	Premenopausal, investigated 2 years post-oophorectomy
5	Postmenopausal, investigated immediately post-oophorectomy
6	6 months post-hysterectomy with conserved ovaries
7	2 years post-hysterectomy with conserved ovaries
8	All patients investigated and treated post-oophorectomy (i.e. Groups 2+3+4+5)
8 A	Control - variable time $<$ 2 years post-oophorectomy
8 B	3 months continuous oestradiol valerate therapy
8 C	6 months continuous oestradiol valerate therapy
8 D	3 months continuous placebo therapy
8 E	3 months continuous conjugated oestrogen therapy

TABLE 3

STATISTICAL COMPARISON BETWEEN THE GROUPS OF PATIENTS INVESTIGATED AND THE OVERALL INCIDENCE (ALL DEGREES OF SEVERITY) OF HOT FLUSHES PER DAY FOR SIGNIFICANT DIFFERENCES

GROUPS COMPARED				STATISTICAL SIGNIFICANCE
GROUP NO.	OVERALL INCIDENCE OF HOT FLUSHES PER DAY	GROUP NO.	OVERALL INCIDENCE OF HOT FLUSHES PER DAY	P <
1	11.1	2	50.0	0.001
1	11.1	3	100.0	0.001
1	11.1	4	61.2	0.001
1	11.1	5	60.0	0.01
1	11.1	6	12.5	..
1	11.1	7	16.7	..
2	50.0	3	100.0	0.01
2	50.0	4	61.2	..
2	50.0	5	60.0	..
2	50.0	6	12.5	0.01
2	50.0	7	16.7	0.01
3	100.0	4	61.2	0.01
3	100.0	5	60.0	0.01
3	100.0	6	12.5	0.001
3	100.0	7	16.7	0.001
4	61.2	5	60.0	..
4	61.2	6	12.5	0.001
4	61.2	7	16.7	0.001
5	60.0	6	12.5	0.001
5	60.0	7	16.7	0.001
6	12.5	7	16.7	..
8 A	68.0	8 B	6.0	0.001
8 A	68.0	8 C	10.0	0.001
8 A	68.0	8 D	66.0	..
8 A	68.0	8 E	14.3	0.001
8 B	6.0	8 C	10.0	..
8 B	6.0	8 D	66.0	0.001
8 B	6.0	8 E	14.3	..
8 C	10.0	8 D	66.0	0.001
8 C	10.0	8 E	14.3	..
8 D	66.0	8 E	14.3	0.001

NOTE: 1. See opposite page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

4.1.2 CLINICAL ATROPHIC VAGINITIS

The incidence of clinical or symptomatic atrophic vaginitis as determined by pain, blood-stained vaginal discharge or dyspareunia, was low. Despite this, a definite relationship ($p < 0.001$) between oestrogen administration and disappearance of symptomatic atrophic vaginitis exists (Table 4). Atrophic vaginitis was present in 20% of the group of 50 oophorectomized patients when first seen. Three months' oestradiol valerate therapy reduced this incidence to 4% and at 6 months there were no patients with clinical atrophic vaginitis. This finding correlates well with the change in cells on vaginal smear (see Section 4.3).

TABLE 4

THE INCIDENCE OF CLINICAL ATROPHIC VAGINITIS IN AN OOPHORECTOMIZED POPULATION AND THE RESPONSE TO OESTROGEN AND PLACEBO THERAPY

CLINICAL ATROPHIC VAGINITIS	CONTROL		3 MONTH OESTRADIOL VALERATE		6 MONTH OESTRADIOL VALERATE		3 MONTH PLACEBO		3 MONTH CONJUGATED OESTROGENS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100
Clinical Atrophic Vaginitis	10	20.0	2	4.0	0	0	0	0	0	0

4.1.3 HEADACHE

The number and incidence of patients complaining of headache is shown in Tables 5 and 6. The response of the oophorectomized females in terms of relief of headache following oestrogen and placebo therapy is summarised in Table 6. The statistical comparison for any significant difference in the incidence of headache of all degrees of severity between the various groups of patients investigated is outlined in Table 7.

No significant relationship has been demonstrated between the incidence of headache and menopause or removal of ovaries. Thus the incidence of patients complaining of headache was similar in the premenopausal group (22.2%) and the group of patients having undergone oophorectomy (22.0%).

The results in the groups investigated after hysterectomy with conservation of ovaries are somewhat incongruous (12.5% at 6 months and 50% at 2 years). The higher incidence in the 2 year group is of possible statistical significance ($p < 0.05$).

Oestradiol valerate administration (Table 6) did not decrease the incidence of this symptom and there was no placebo effect. Administration of conjugated oestrogen after placebo reduced the incidence of headache from 32.0% to 14.3% but this was only of possible significance ($p < 0.05$). In this respect, therefore, the effect of conjugated equine oestrogen was possibly superior to that of oestradiol valerate ($p < 0.05$).

Headache is thus shown to be a symptom unrelated to oophorectomy but possibly relieved by conjugated oestrogen therapy.

TABLE 5

THE INCIDENCE AND SEVERITY OF HEADACHE IN THE GROUPS OF PATIENTS INVESTIGATED

HEADACHE	NORMAL		POST-OOPHORECTOMY								CONSERVED OVARIES			
	PREMENOPAUSAL		PREMENOPAUSAL						POSTMENOPAUSAL		POST-HYSTERECTOMY			
			Immediate		6 months		2 years		Immediate		6 months		2 years	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients studied	9	100	14	100	13	100	18	100	5	100	8	100	18	100
Headache - Absent	7	77.8	11	78.6	11	84.6	13	72.2	4	80.0	7	87.5	9	50.0
Mild	2	22.2	2	14.3	0	0	2	11.1	1	20.0	1	12.5	6	33.3
Moderate	0	0	1	7.1	2	15.4	3	16.7	0	0	0	0	2	11.1
Severe	0	0	0	0	0	0	0	0	0	0	0	0	1	5.6

TABLE 6

THE INCIDENCE OF HEADACHE IN AN OOPHORECTOMIZED POPULATION AND THE EFFECT OF OESTROGEN AND PLACEBO THERAPY

HEADACHE	CONTROL		3 MONTH OESTRADIOL VALERATE		6 MONTH OESTRADIOL VALERATE		3 MONTH PLACEBO		3 MONTH CONJUGATED OESTROGENS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100
Absent	39	78.0	38	76.0	36	72.0	34	68.0	42	85.7
Mild	5	10.0	9	18.0	11	22.0	13	26.0	5	10.2
Moderate	6	12.0	3	6.0	3	6.0	2	4.0	1	4.1
Severe	0	0	0	0	0	0	1	2.0	0	0

EXPLANATION OF GROUP NUMBERS DESCRIBED
IN TABLE OF STATISTICAL COMPARISON

GROUP NO.	DESCRIPTION OF GROUP OF PATIENTS
1	Normal premenopausal
2	Premenopausal, investigated immediately post-oophorectomy
3	Premenopausal, investigated 6 months post-oophorectomy
4	Premenopausal, investigated 2 years post-oophorectomy
5	Postmenopausal, investigated immediately post-oophorectomy
6	6 months post-hysterectomy with conserved ovaries
7	2 years post-hysterectomy with conserved ovaries
8	All patients investigated and treated post-oophorectomy (i.e. Groups 2+3+4+5)
8 A	Control - variable time < 2 years post-oophorectomy
8 B	3 months continuous oestradiol valerate therapy
8 C	6 months continuous oestradiol valerate therapy
8 D	3 months continuous placebo therapy
8 E	3 months continuous conjugated oestrogen therapy

TABLE 7

STATISTICAL COMPARISON BETWEEN THE GROUPS OF PATIENTS INVESTIGATED AND THE OVERALL INCIDENCE (ALL DEGREES OF SEVERITY) OF HEADACHE FOR SIGNIFICANT DIFFERENCES

GROUPS COMPARED				STATISTICAL SIGNIFICANCE
GROUP NO.	OVERALL INCIDENCE OF HEADACHE	GROUP NO.	OVERALL INCIDENCE OF HEADACHE	P <
1	22.2	2	21.4	..
1	22.2	3	15.4	..
1	22.2	4	27.8	..
1	22.2	5	20.0	..
1	22.2	6	12.5	..
1	22.2	7	50.0	0.05
2	21.4	3	15.4	..
2	21.4	4	27.8	..
2	21.4	5	20.0	..
2	21.4	6	12.5	..
2	21.4	7	50.0	0.05
3	15.4	4	27.8	..
3	15.4	5	20.0	..
3	15.4	6	12.5	..
3	15.4	7	50.0	0.01
4	27.8	5	20.0	..
4	27.8	6	12.5	..
4	27.8	7	50.0	0.05
5	20.0	6	12.5	..
5	20.0	7	50.0	0.05
6	12.5	7	50.0	0.01
8 A	22.0	8 B	24.0	..
8 A	22.0	8 C	28.0	..
8 A	22.0	8 D	32.0	..
8 A	22.0	8 E	14.3	..
8 B	24.0	8 C	28.0	..
8 B	24.0	8 D	32.0	..
8 B	24.0	8 E	14.3	..
8 C	28.0	8 D	32.0	..
8 C	28.0	8 E	14.3	0.05
8 D	32.0	8 E	14.3	0.05

NOTE: 1. See opposite page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

4.1.4. PALPITATIONS

The incidence of palpitations in all the groups studied is shown in Tables 8 and 9. The response of oophorectomized females to oestrogen and placebo therapy is also listed in Table 9. Table 10 summarises the statistical comparison between the groups of patients investigated in terms of the incidence of palpitations.

No premenopausal patients complained of palpitations. The incidence of this symptom was similar (20% to 38.9%) in all the other groups of patients prior to any treatment. Thus oophorectomy is not shown to result in an increased incidence of palpitations.

The response of the oophorectomized group of patients to treatment was of possible significance. Oestradiol valerate therapy reduced the incidence of palpitations from 30.0% to 14.0% ($p < 0.05$) over a period of 6 months. However, single-blind crossover to placebo therapy resulted in a further drop in this incidence to 8.0% and the subsequent cross-over to conjugated oestrogen therapy showed no significant change. The overall interpretation of this behaviour, despite the low degree of statistical significance, is that the response of this symptom to treatment is purely a placebo effect.

Hence oestrogen deprivation following bilateral oophorectomy did not result in an increased incidence of palpitations; similarly, exogenous oestrogen administration per se was of no value in the treatment of this symptom.

TABLE 8

INCIDENCE OF PALPITATIONS IN THE GROUPS INVESTIGATED

PALPITATIONS	NORMAL		POST-OOPHORECTOMY								CONSERVED OVARIES			
	PREMENOPAUSAL		PREMENOPAUSAL						POSTMENOPAUSAL		POST-HYSTERECTOMY			
			Immediate		6 months		2 years		Immediate		6 months		2 years	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients studied	9	100	14	100	13	100	18	100	5	100	8	100	18	100
Palpitations -														
Absent	9	100	11	78.6	9	69.2	11	61.1	4	80.0	6	75.0	14	77.8
Mild	0	0	2	14.3	2	15.4	4	22.2	1	20.0	2	25.0	3	16.6
Moderate	0	0	1	7.1	2	15.4	2	11.1	0	0	0	0	1	5.6
Severe	0	0	0	0	0	0	1	5.6	0	0	0	0	0	0

TABLE 9

THE INCIDENCE OF PALPITATIONS IN AN OOPHORECTOMIZED POPULATION AND THE EFFECT OF OESTROGEN AND PLACEBO THERAPY

PALPITATIONS	CONTROL		3 MONTH OESTRADIOL VALERATE		6 MONTH OESTRADIOL VALERATE		3 MONTH PLACEBO		3 MONTH CONJUGATED OESTROGENS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100
Absent	35	70.0	41	82.0	43	86.0	46	92.0	44	89.8
Mild	9	18.0	7	14.0	5	10.0	3	6.0	3	6.1
Moderate	5	10.0	2	4.0	2	4.0	1	2.0	2	4.1
Severe	1	2.0	0	0	0	0	0	0	0	0

EXPLANATION OF GROUP NUMBERS DESCRIBED IN TABLE OF STATISTICAL COMPARISON

GROUP NO.	DESCRIPTION OF GROUP OF PATIENTS
1	Normal premenopausal
2	Premenopausal, investigated immediately post-oophorectomy
3	Premenopausal, investigated 6 months post-oophorectomy
4	Premenopausal, investigated 2 years post-oophorectomy
5	Postmenopausal, investigated immediately post-oophorectomy
6	6 months post-hysterectomy with conserved ovaries
7	2 years post-hysterectomy with conserved ovaries
8	All patients investigated and treated post-oophorectomy (i.e. Groups 2+3+4+5)
8 A	Control - variable time < 2 years post-oophorectomy
8 B	3 months continuous oestradiol valerate therapy
8 C	6 months continuous oestradiol valerate therapy
8 D	3 months continuous placebo therapy
8 E	3 months continuous conjugated oestrogen therapy

TABLE 10

STATISTICAL COMPARISON BETWEEN THE GROUPS OF PATIENTS INVESTIGATED AND THE OVERALL INCIDENCE (ALL DEGREES OF SEVERITY) OF PALPITATIONS FOR SIGNIFICANT DIFFERENCES

GROUPS COMPARED				STATISTICAL SIGNIFICANCE
GROUP NO.	OVERALL INCIDENCE OF PALPITATIONS	GROUP NO.	OVERALL INCIDENCE OF PALPITATIONS	P <
1	0	2	21.4	0.001
1	0	3	30.8	0.001
1	0	4	38.9	0.001
1	0	5	20.0	0.01
1	0	6	25.0	0.001
1	0	7	22.2	0.001
2	21.4	3	30.8	..
2	21.4	4	38.9	..
2	21.4	5	20.0	..
2	21.4	6	25.0	..
2	21.4	7	22.2	..
3	30.8	4	38.9	..
3	30.8	5	20.0	..
3	30.8	6	25.0	..
3	30.8	7	22.2	..
4	38.9	5	20.0	..
4	38.9	6	25.0	..
4	38.9	7	22.2	..
5	20.0	6	25.0	..
5	20.0	7	22.2	..
6	25.0	7	22.2	..
8 A	30.0	8 B	18.0	0.05
8 A	30.0	8 C	14.0	0.05
8 A	30.0	8 D	8.0	0.05
8 A	30.0	8 E	10.2	0.05
8 B	18.0	8 C	14.0	..
8 B	18.0	8 D	8.0	..
8 B	18.0	8 E	10.2	..
8 C	14.0	8 D	8.0	..
8 C	14.0	8 E	10.2	..
8 D	8.0	8 E	10.2	..

NOTE: 1. See opposite page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

4.1.5 ANGINA PECTORIS

The number and incidence of patients complaining of angina pectoris is outlined in Tables 11 and 12. The effect of oestrogen and placebo therapy on oophorectomized patients is shown in Table 12. The statistical comparison of the incidence of angina pectoris between the various groups investigated is summarised in Table 13.

Angina pectoris becomes a significant complaint after the procedure of bilateral oophorectomy in the premenopausal female ($p < 0.001$) (See Table 13). Oophorectomy performed on the postmenopausal female does not significantly affect the incidence of this symptom in comparison with the normal premenopausal group of patients. Conservation of ovaries protects against the development of angina pectoris in both the 6 month groups ($p < 0.001$) and the 2 year groups ($p < 0.001$).

The response of angina pectoris to oestrogen and placebo therapy is similar to that described for palpitations under Section 4.1.4. Thus the slight response to therapy indicates a placebo effect only.

Angina pectoris is thus shown to be a symptom related to oophorectomy but unrelieved by oestrogen therapy. Nevertheless, some relief of angina pectoris occurs with placebo therapy.

TABLE 11

INCIDENCE OF ANGINA PECTORIS IN THE GROUPS OF PATIENTS INVESTIGATED

ANGINA PECTORIS	NORMAL		POST-OOPHORECTOMY								CONSERVED OVARIES			
	PREMENOPAUSAL		PREMENOPAUSAL						POSTMENOPAUSAL		POST-HYSTERECTOMY			
			Immediate		6 months		2 years		Immediate		6 months		2 years	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients studied	9	100	14	100	13	100	18	100	5	100	8	100	18	100
Angina - Absent	9	100	12	85.7	9	69.2	13	72.2	5	100	8	100	18	100
Present	0	0	2	14.3	4	30.8	5	27.8	0	0	0	0	0	0

TABLE 12

THE EFFECT OF OESTROGEN AND PLACEBO THERAPY ON THE INCIDENCE OF ANGINA PECTORIS IN AN OOPHORECTOMIZED POPULATION

ANGINA PECTORIS	CONTROL		3 MONTH OESTRADIOL VALERATE		6 MONTH OESTRADIOL VALERATE		3 MONTH PLACEBO		3 MONTH CONJUGATED OESTROGENS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100
Absent	39	78.0	44	88.0	46	92.0	46	92.0	47	95.9
Present	11	22.0	6	12.0	4	8.0	4	8.0	2	4.1

EXPLANATION OF GROUP NUMBERS DESCRIBED IN TABLE OF STATISTICAL COMPARISON

GROUP NO.	DESCRIPTION OF GROUP OF PATIENTS
1	Normal premenopausal
2	Premenopausal, investigated immediately post-oophorectomy
3	Premenopausal, investigated 6 months post-oophorectomy
4	Premenopausal, investigated 2 years post-oophorectomy
5	Postmenopausal, investigated immediately post-oophorectomy
6	6 months post-hysterectomy with conserved ovaries
7	2 years post-hysterectomy with conserved ovaries
8	All patients investigated and treated post-oophorectomy (i.e. Groups 2+3+4+5)
8 A	Control - variable time < 2 years post-oophorectomy
8 B	3 months continuous oestradiol valerate therapy
8 C	6 months continuous oestradiol valerate therapy
8 D	3 months continuous placebo therapy
8 E	3 months continuous conjugated oestrogen therapy

TABLE 13

STATISTICAL COMPARISON BETWEEN THE GROUPS OF PATIENTS INVESTIGATED AND THE OVERALL INCIDENCE OF ANGINA PECTORIS FOR SIGNIFICANT DIFFERENCES

GROUPS COMPARED				STATISTICAL SIGNIFICANCE
GROUP NO.	OVERALL INCIDENCE OF ANGINA PECTORIS	GROUP NO.	OVERALL INCIDENCE OF ANGINA PECTORIS	P <
1	0	2	14.3	0.01
1	0	3	30.8	0.001
1	0	4	27.8	0.001
1	0	5	0	..
1	0	6	0	..
1	0	7	0	..
2	14.3	3	30.8	..
2	14.3	4	27.8	..
2	14.3	5	0	0.01
2	14.3	6	0	0.01
2	14.3	7	0	0.01
3	30.8	4	27.8	..
3	30.8	5	0	0.001
3	30.8	6	0	0.001
3	30.8	7	0	0.001
4	27.8	5	0	0.001
4	27.8	6	0	0.001
4	27.8	7	0	0.001
5	0	6	0	..
5	0	7	0	..
6	0	7	0	..
8 A	22.0	8 B	12.0	..
8 A	22.0	8 C	8.0	0.05
8 A	22.0	8 D	8.0	0.05
8 A	22.0	8 E	4.1	0.05
8 B	12.0	8 C	8.0	..
8 B	12.0	8 D	8.0	..
8 B	12.0	8 E	4.1	..
8 C	8.0	8 D	8.0	..
8 C	8.0	8 E	4.1	..
8 D	8.0	8 E	4.1	..

NOTE: 1. See opposite page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

4.1.6 INSOMNIA

The number and incidence of patients complaining of insomnia is outlined in Tables 14 and 15. The effect of oestrogen and placebo therapy on insomnia in oophorectomized females is shown in Table 15. The statistical comparison between the groups studied for the significance of the incidence of insomnia is summarised in Table 16.

Insomnia is shown to be a common symptom in all groups of patients except the normal premenopausal group. There is only a possible statistical difference between the oophorectomized and non-oophorectomized patients ($p < 0.05$). It is thus not possible to draw any conclusions in this respect.

However, the oophorectomized groups receiving oestrogen and placebo therapy show a highly significant response to treatment. The effect of treatment is a placebo one similar to that described under section 4.1.4, but of much greater statistical significance. Oestradiol valerate therapy for 6 months results in a reduction of the incidence of insomnia from 52.0% to 26.0% ($p < 0.01$). Single blind cross-over to placebo therapy and subsequent cross-over to conjugated oestrogens resulted in little change in the incidence of this symptom.

TABLE 14

THE INCIDENCE OF INSOMNIA IN PATIENTS OF THE SEVERAL GROUPS STUDIED

INSOMNIA	NORMAL		POST-OOPHORECTOMY								CONSERVED OVARIES			
	PREMENOPAUSAL		PREMENOPAUSAL						POSTMENOPAUSAL		POST-HYSTERECTOMY			
			Immediate		6 months		2 years		Immediate		6 months		2 years	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients studied	9	100	14	100	13	100	18	100	5	100	8	100	18	100
Insomnia -														
Absent	9	100	7	50.0	5	38.5	10	55.6	2	40.0	6	75.0	12	66.7
Present	0	0	7	50.0	8	61.5	8	44.4	3	60.0	2	25.0	6	33.3

TABLE 15

THE EFFECT OF OESTROGEN AND PLACEBO THERAPY ON THE INCIDENCE OF INSOMNIA IN AN OOPHORECTOMIZED POPULATION

INSOMNIA	CONTROL		3 MONTH OESTRADIOL VALERATE		6 MONTH OESTRADIOL VALERATE		3 MONTH PLACEBO		3 MONTH CONJUGATED OESTROGENS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100
Absent	24	48.0	41	82.0	37	74.0	41	82.0	43	87.8
Present	26	52.0	9	18.0	13	26.0	9	18.0	6	12.2

EXPLANATION OF GROUP NUMBERS DESCRIBED
IN TABLE OF STATISTICAL COMPARISON

GROUP NO.	DESCRIPTION OF GROUP OF PATIENTS
1	Normal premenopausal
2	Premenopausal, investigated immediately post-oophorectomy
3	Premenopausal, investigated 6 months post-oophorectomy
4	Premenopausal, investigated 2 years post-oophorectomy
5	Postmenopausal, investigated immediately post-oophorectomy
6	6 months post-hysterectomy with conserved ovaries
7	2 years post-hysterectomy with conserved ovaries
8	All patients investigated and treated post-oophorectomy (i.e. Groups 2+3+4+5)
8 A	Control - variable time < 2 years post-oophorectomy
8 B	3 months continuous oestradiol valerate therapy
8 C	6 months continuous oestradiol valerate therapy
8 D	3 months continuous placebo therapy
8 E	3 months continuous conjugated oestrogen therapy

TABLE 16

STATISTICAL COMPARISON BETWEEN THE GROUPS OF PATIENTS INVESTIGATED AND THE OVERALL INCIDENCE OF INSOMNIA FOR SIGNIFICANT DIFFERENCES

GROUPS COMPARED				STATISTICAL SIGNIFICANCE
GROUP NO.	OVERALL INCIDENCE OF INSOMNIA	GROUP NO.	OVERALL INCIDENCE OF INSOMNIA	P <
1	0	2	50.0	0.001
1	0	3	61.5	0.001
1	0	4	44.4	0.001
1	0	5	60.0	0.001
1	0	6	25.0	0.001
1	0	7	33.3	0.001
2	50.0	3	61.5	..
2	50.0	4	44.4	..
2	50.0	5	60.0	..
2	50.0	6	25.0	0.05
2	50.0	7	33.3	0.05
3	61.5	4	44.4	..
3	61.5	5	60.0	..
3	61.5	6	25.0	0.01
3	61.5	7	33.3	0.01
4	44.4	5	60.0	..
4	44.4	6	25.0	0.05
4	44.4	7	33.3	..
5	60.0	6	25.0	0.01
5	60.0	7	33.3	0.05
6	25.0	7	33.3	..
8 A	52.0	8 B	18.0	0.001
8 A	52.0	8 C	26.0	0.01
8 A	52.0	8 D	18.0	0.001
8 A	52.0	8 E	12.2	0.001
8 B	18.0	8 C	26.0	..
8 B	18.0	8 D	18.0	..
8 B	18.0	8 E	12.2	..
8 C	26.0	8 D	18.0	..
8 C	26.0	8 E	12.2	0.05
8 D	18.0	8 E	12.2	..

NOTE: 1. See opposite page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

4.1.7 IRRITABILITY

The number and incidence of patients complaining of irritability is shown in Tables 17 and 18. The effect of oestrogen and placebo therapy on irritability in oophorectomized females is listed in Table 17. The statistical comparison between the groups studied for significant differences in the incidence of irritability is summarised in Table 19.

It is clearly shown that irritability is not an effect of bilateral oophorectomy. No significant relationship exists between any of the oophorectomized and non-oophorectomized groups.

Oestrogen and placebo therapy to oophorectomized females have essentially a placebo effect on the symptom of irritability, as explained under Section 4.1.4.

However, conjugated oestrogen therapy did produce more than just a placebo effect. Comparison of the control group of oophorectomized females to the conjugated oestrogen treated group showed incidences of 38.0% and 8.2% respectively ($p < 0.01$). Comparison of the 3 month oestradiol valerate group to the 3 month conjugated oestrogen group showed this difference still to be of possible significance ($p < 0.05$) and this relationship is again confirmed by comparing placebo therapy to conjugated oestrogen therapy ($p < 0.05$).

The conclusion to be drawn from these findings is that irritability is not a response of oophorectomy and is relieved essentially by placebo therapy. The fact that conjugated oestrogens have some ability to relieve this symptom may be related to other effects of conjugated oestrogen, notably the mood elevating effect, and will be discussed under Section 5.1.

TABLE 17

NUMBER AND INCIDENCE OF PATIENTS DEMONSTRATING THE SYMPTOM OF IRRITABILITY

IRRITABILITY	NORMAL		POST-OOPHORECTOMY								CONSERVED OVARIES			
	PREMENOPAUSAL		PREMENOPAUSAL						POSTMENOPAUSAL		POST-HYSTERECTOMY			
			Immediate		6 months		2 years		Immediate		6 months		2 years	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients studied	9	100	14	100	13	100	18	100	5	100	8	100	18	100
Irritability -														
Absent	6	66.7	9	64.3	8	61.5	12	66.7	2	40.0	5	62.5	13	72.2
Mild	3	33.3	1	7.1	3	23.1	6	33.3	2	40.0	1	12.5	4	22.2
Moderate	0	0	3	21.4	2	15.4	0	0	1	20.0	2	25.0	1	5.6
Severe	0	0	1	7.1	0	0	0	0	0	0	0	0	0	0

TABLE 18

THE EFFECT OF OESTROGEN AND PLACEBO THERAPY ON THE SYMPTOM OF IRRITABILITY IN OOPHORECTOMIZED FEMALES

IRRITABILITY	CONTROL		3 MONTH OESTRADIOL VALERATE		6 MONTH OESTRADIOL VALERATE		3 MONTH PLACEBO		3 MONTH CONJUGATED OESTROGENS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100
Absent	31	62.0	40	80.0	40	80.0	39	78.0	45	91.8
Mild	12	24.0	8	16.0	8	16.0	7	14.0	3	6.2
Moderate	6	12.0	2	4.0	2	4.0	4	8.0	1	2.0
Severe	1	2.0	0	0	0	0	0	0	0	0

EXPLANATION OF GROUP NUMBERS DESCRIBED
IN TABLE OF STATISTICAL COMPARISON

GROUP NO.	DESCRIPTION OF GROUP OF PATIENTS
1	Normal premenopausal
2	Premenopausal, investigated immediately post-oophorectomy
3	Premenopausal, investigated 6 months post-oophorectomy
4	Premenopausal, investigated 2 years post-oophorectomy
5	Postmenopausal, investigated immediately post-oophorectomy
6	6 months post-hysterectomy with conserved ovaries
7	2 years post-hysterectomy with conserved ovaries
8	All patients investigated and treated post-oophorectomy (i.e. Groups 2+3+4+5)
8 A	Control - variable time < 2 years post-oophorectomy
8 B	3 months continuous oestradiol valerate therapy
8 C	6 months continuous oestradiol valerate therapy
8 D	3 months continuous placebo therapy
8 E	3 months continuous conjugated oestrogen therapy

TABLE 19

STATISTICAL COMPARISON BETWEEN THE GROUPS OF PATIENTS INVESTIGATED AND THE OVERALL INCIDENCE (ALL DEGREES OF SEVERITY) OF IRRITABILITY FOR SIGNIFICANT DIFFERENCES

GROUPS COMPARED				STATISTICAL SIGNIFICANCE
GROUP NO.	OVERALL INCIDENCE OF IRRITABILITY	GROUP NO.	OVERALL INCIDENCE OF IRRITABILITY	P <
1	33.3	2	35.6	..
1	33.3	3	38.5	..
1	33.3	4	33.3	..
1	33.3	5	60.0	0.05
1	33.3	6	37.5	..
1	33.3	7	27.8	..
2	35.6	3	38.5	..
2	35.6	4	33.3	..
2	35.6	5	60.0	0.05
2	35.6	6	37.5	..
2	35.6	7	27.8	..
3	38.5	4	33.3	..
3	38.5	5	60.0	..
3	38.5	6	37.5	..
3	38.5	7	27.8	..
4	33.3	5	60.0	0.05
4	33.3	6	37.5	..
4	33.3	7	27.8	..
5	60.0	6	37.5	..
5	60.0	7	27.8	0.01
6	37.5	7	27.8	..
8 A	38.0	8 B	20.0	0.05
8 A	38.0	8 C	20.0	0.05
8 A	38.0	8 D	22.0	0.05
8 A	38.0	8 E	8.2	0.01
8 B	20.0	8 C	20.0	..
8 B	20.0	8 D	22.0	..
8 B	20.0	8 E	8.2	0.05
8 C	20.0	8 D	22.0	..
8 C	20.0	8 E	8.2	0.05
8 D	22.0	8 E	8.2	0.05

NOTE: 1. See opposite page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

4.1.8 DEPRESSION

The number and incidence of patients affected by depression in the various groups investigated is shown in Tables 20 and 21. The effect of oestrogen and placebo therapy on depression in oophorectomized females is also shown in Table 21. The statistical comparison for significant differences between the groups studied in the incidence of depression is summarised in Table 22.

Oophorectomy appears to result in a proportion of patients developing depression, although the results generally are of possible statistical significance only. The premenopausal and non-oophorectomized groups presented an overall incidence of depression of 22.2% and 37.5%. The incidence ranged from 46.2% to 64.3% in the four post-oophorectomy groups studied.

Oestrogen and placebo therapy produced a statistically highly significant response ($p < 0.001$). The control post-oophorectomy group had an overall incidence of depression of 56.0%. Oestradiol valerate therapy reduced the incidence to 30.0% at 3 months ($p < 0.01$) and to 24.0% at 6 months ($p < 0.01$). Single blind cross-over to placebo further reduced this incidence to 20.0% ($p < 0.001$) and the final cross-over to conjugated oestrogen resulted in a further drop to 10.2% ($p < 0.001$). This progressive fall in incidence of depression through the year of treatment can be explained on a placebo basis.

These observations seem to indicate that the operation of oophorectomy results in a significant incidence of depression which apparently develops on a psychological basis as demonstrated by the highly significant response to placebo therapy.

TABLE 20

INCIDENCE OF DEPRESSION IN THE GROUPS OF PATIENTS INVESTIGATED

DEPRESSION	NORMAL		POST-OOPHORECTOMY								CONSERVED OVARIES			
	PREMENOPAUSAL		PREMENOPAUSAL						POSTMENOPAUSAL		POST-HYSTERECTOMY			
			Immediate		6 months		2 years		Immediate		6 months		2 years	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients studied	9	100	14	100	13	100	18	100	5	100	8	100	18	100
Depression -														
Absent	7	77.8	5	35.7	7	53.8	8	44.5	2	40.0	5	62.5	12	66.6
Mild	2	22.2	4	28.6	4	30.8	6	33.3	2	40.0	0	0	3	16.7
Moderate - Severe	0	0	5	35.7	2	15.4	4	22.2	1	20.0	3	37.5	3	16.7

TABLE 21

THE EFFECT OF OESTROGEN AND PLACEBO THERAPY ON THE SYMPTOM OF DEPRESSION IN OOPHORECTOMIZED FEMALES

DEPRESSION	CONTROL		3 MONTH OESTRADIOL VALERATE		6 MONTH OESTRADIOL VALERATE		3 MONTH PLACEBO		3 MONTH CONJUGATED OESTROGENS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100
Absent	22	44.0	35	70.0	38	76.0	40	80.0	44	89.8
Mild	16	32.0	11	22.0	9	18.0	6	12.0	3	6.1
Moderate/Severe	12	24.0	4	8.0	3	6.0	4	8.0	2	4.1

EXPLANATION OF GROUP NUMBERS DESCRIBED
IN TABLE OF STATISTICAL COMPARISON

GROUP NO.	DESCRIPTION OF GROUP OF PATIENTS
1	Normal premenopausal
2	Premenopausal, investigated immediately post-oophorectomy
3	Premenopausal, investigated 6 months post-oophorectomy
4	Premenopausal, investigated 2 years post-oophorectomy
5	Postmenopausal, investigated immediately post-oophorectomy
6	6 months post-hysterectomy with conserved ovaries
7	2 years post-hysterectomy with conserved ovaries
8	All patients investigated and treated post-oophorectomy (i.e. Groups 2+3+4+5)
8 A	Control - variable time < 2 years post-oophorectomy
8 B	3 months continuous oestradiol valerate therapy
8 C	6 months continuous oestradiol valerate therapy
8 D	3 months continuous placebo therapy
8 E	3 months continuous conjugated oestrogen therapy

TABLE 22

STATISTICAL COMPARISON BETWEEN THE GROUPS OF PATIENTS INVESTIGATED AND THE OVERALL INCIDENCE (ALL DEGREES OF SEVERITY) OF DEPRESSION FOR SIGNIFICANT DIFFERENCES

GROUPS COMPARED				STATISTICAL SIGNIFICANCE
GROUP NO.	OVERALL INCIDENCE OF DEPRESSION	GROUP NO.	OVERALL INCIDENCE OF DEPRESSION	P <
1	22.2	2	64.3	0.01
1	22.2	3	46.2	0.05
1	22.2	4	55.5	0.01
1	22.2	5	60.0	0.01
1	22.2	6	37.5	..
1	22.2	7	33.4	..
2	64.3	3	46.2	..
2	64.3	4	55.5	..
2	64.3	5	60.0	..
2	64.3	6	37.5	0.05
2	64.3	7	33.4	0.05
3	46.2	4	55.5	..
3	46.2	5	60.0	..
3	46.2	6	37.5	..
3	46.2	7	33.4	..
4	55.5	5	60.0	..
4	55.5	6	37.5	..
4	55.5	7	33.4	0.05
5	60.0	6	37.5	0.05
5	60.0	7	33.4	0.05
6	37.5	7	33.4	..
8 A	56.0	8 B	30.0	0.01
8 A	56.0	8 C	24.0	0.01
8 A	56.0	8 D	20.0	0.001
8 A	56.0	8 E	10.2	0.001
8 B	30.0	8 C	24.0	..
8 B	30.0	8 D	20.0	..
8 B	30.0	8 E	10.2	0.05
8 C	24.0	8 D	20.0	..
8 C	24.0	8 E	10.2	0.05
8 D	20.0	8 E	10.2	..

NOTE: 1. See opposite page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

4.1.9 LOW BACKACHE

The number and incidence of patients affected by low backache in the various groups investigated is shown in Tables 23 and 24. The effect of oestrogen and placebo therapy on low backache in oophorectomized females is also shown in Table 24. The statistical comparison for significant differences between the groups studied in the incidence of backache is summarised in Table 25.

Despite a few relationships of doubtful significance there would seem to be only one interpretation of these results, namely that backache is not a factor related to oophorectomy and similarly cannot be relieved by oestrogen therapy. Moreover, it is considered significant that low backache, being more real a symptom than, for example, insomnia or depression, showed no placebo response to therapy.

TABLE 23

THE INCIDENCE OF LOW BACKACHE IN THE GROUPS OF PATIENTS STUDIED

LOW BACKACHE	NORMAL		POST-OOPHORECTOMY								CONSERVED OVARIES POST-HYSTERECTOMY			
	PREMENOPAUSAL		PREMENOPAUSAL						POSTMENOPAUSAL					
			Immediate		6 months		2 years		Immediate		6 months		2 years	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients studied	9	100	14	100	13	100	18	100	5	100	8	100	18	100
Low Backache														
Absent	6	66.7	12	85.7	7	53.8	11	61.1	4	80.0	7	87.5	10	55.6
Present	3	33.3	2	14.3	6	46.2	7	38.9	1	10.0	1	12.5	8	44.4

TABLE 24

THE EFFECT OF OESTROGEN AND PLACEBO THERAPY ON THE SYMPTOM OF LOW BACKACHE IN OOPHORECTOMIZED FEMALES

LOW BACKACHE	CONTROL		3 MONTH OESTRADIOL VALERATE		6 MONTH OESTRADIOL VALERATE		3 MONTH PLACEBO		3 MONTH CONJUGATED OESTROGENS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100
Absent	34	68.0	36	72.0	36	72.0	35	70.0	37	75.5
Present	16	32.0	14	28.0	14	28.0	15	30.0	12	24.5

EXPLANATION OF GROUP NUMBERS DESCRIBED
IN TABLE OF STATISTICAL COMPARISON

GROUP NO.	DESCRIPTION OF GROUP OF PATIENTS
1	Normal premenopausal
2	Premenopausal, investigated immediately post-oophorectomy
3	Premenopausal, investigated 6 months post-oophorectomy
4	Premenopausal, investigated 2 years post-oophorectomy
5	Postmenopausal, investigated immediately post-oophorectomy
6	6 months post-hysterectomy with conserved ovaries
7	2 years post-hysterectomy with conserved ovaries
8	All patients investigated and treated post-oophorectomy (i.e. Groups 2+3+4+5)
8 A	Control - variable time $<$ 2 years post-oophorectomy
8 B	3 months continuous oestradiol valerate therapy
8 C	6 months continuous oestradiol valerate therapy
8 D	3 months continuous placebo therapy
8 E	3 months continuous conjugated oestrogen therapy

TABLE 25

STATISTICAL COMPARISON BETWEEN THE GROUPS OF PATIENTS INVESTIGATED AND THE OVERALL INCIDENCE OF LOW BACKACHE FOR SIGNIFICANT DIFFERENCES

GROUPS COMPARED				STATISTICAL SIGNIFICANCE
GROUP NO.	OVERALL INCIDENCE OF LOW BACKACHE	GROUP NO.	OVERALL INCIDENCE OF LOW BACKACHE	P <
1	33.3	2	14.3	..
1	33.3	3	46.2	..
1	33.3	4	38.9	..
1	33.3	5	10.0	..
1	33.3	6	12.5	..
1	33.3	7	44.4	..
2	14.3	3	46.2	0.05
2	14.3	4	38.9	0.05
2	14.3	5	10.0	..
2	14.3	6	12.5	..
2	14.3	7	44.4	0.05
3	46.2	4	38.9	..
3	46.2	5	10.0	0.05
3	46.2	6	12.5	0.05
3	46.2	7	44.4	..
4	38.9	5	10.0	0.05
4	38.9	6	12.5	0.05
4	38.9	7	44.4	..
5	10.0	6	12.5	..
5	10.0	7	44.4	0.05
6	12.5	7	44.4	0.05
8 A	32.0	8 B	28.0	..
8 A	32.0	8 C	28.0	..
8 A	32.0	8 D	30.0	..
8 A	32.0	8 E	24.5	..
8 B	28.0	8 C	28.0	..
8 B	28.0	8 D	30.0	..
8 B	28.0	8 E	24.5	..
8 C	28.0	8 D	30.0	..
8 C	28.0	8 E	24.5	..
8 D	30.0	8 E	24.5	..

NOTE: 1. See opposite page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

4.1.10 LIBIDO

The number and incidence of patients complaining of severely decreased or absent libido in the various groups investigated is shown in Tables 26 and 27. The effect of oestrogen and placebo therapy on libido in oophorectomized females is also shown in Table 27. The statistical comparison for significant differences between the groups studied in the incidence of absent libido is summarised in Table 28.

No females in the normal premenopausal group complained of absent libido. On the other hand there was a high incidence of this complaint in all the groups of patients having undergone surgery. The strikingly high incidence of this symptom in the immediate postoperative groups (Groups 2 and 5) is not unexpected in view of the proximity to surgery. Nevertheless, the groups investigated 6 months and 2 years postoperatively (Groups 3, 4, 6 and 7) presented an incidence of absent libido varying from 25.0% to 44.0% and these figures showed a statistically highly significant difference ($p < 0.001$) to the normal premenopausal patients. Furthermore, there was no effect on this symptom by oestrogen or placebo therapy.

The interpretation of these results would appear to be that the operation of hysterectomy per se, irrespective of whether the ovaries are removed or not, is associated with a significant reduction of libido. Moreover, oestrogen therapy is shown to be of no benefit in the treatment of decreased or absent libido.

TABLE 26

THE INCIDENCE OF NORMAL OR ABSENT LIBIDO IN THE GROUPS STUDIED

LIBIDO	NORMAL		POST-OOPHORECTOMY								CONSERVED OVARIES			
	PREMENOPAUSAL		PREMENOPAUSAL						POSTMENOPAUSAL		POST-HYSTERECTOMY			
			Immediate		6 months		2 years		Immediate		6 months		2 years	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients studied	9	100	14	100	13	100	18	100	5	100	8	100	18	100
Libido -														
Normal	9	100	4	28.6	8	61.5	10	55.6	1	20.0	6	75.0	12	66.7
Absent	0	0	10	71.4	5	38.5	8	44.4	4	80.0	2	25.0	6	33.3

TABLE 27

THE EFFECTS OF OESTROGEN AND PLACEBO THERAPY ON LIBIDO OF OOPHORECTOMIZED FEMALES

LIBIDO	CONTROL		3 MONTH OESTRADIOL VALERATE		6 MONTH OESTRADIOL VALERATE		3 MONTH PLACEBO		3 MONTH CONJUGATED OESTROGENS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100
Normal	24	48.0	26	52.0	26	52.0	23	46.0	22	44.9
Absent	26	52.0	24	48.0	24	48.0	27	54.0	27	55.1

EXPLANATION OF GROUP NUMBERS DESCRIBED
IN TABLE OF STATISTICAL COMPARISON

GROUP NO.	DESCRIPTION OF GROUP OF PATIENTS
1	Normal premenopausal
2	Premenopausal, investigated immediately post-oophorectomy
3	Premenopausal, investigated 6 months post-oophorectomy
4	Premenopausal, investigated 2 years post-oophorectomy
5	Postmenopausal, investigated immediately post-oophorectomy
6	6 months post-hysterectomy with conserved ovaries
7	2 years post-hysterectomy with conserved ovaries
8	All patients investigated and treated post-oophorectomy (i.e. Groups 2+3+4+5)
8 A	Control - variable time $<$ 2 years post-oophorectomy
8 B	3 months continuous oestradiol valerate therapy
8 C	6 months continuous oestradiol valerate therapy
8 D	3 months continuous placebo therapy
8 E	3 months continuous conjugated oestrogen therapy

TABLE 28

STATISTICAL COMPARISON BETWEEN THE GROUPS OF PATIENTS INVESTIGATED AND THE OVERALL INCIDENCE OF ABSENT LIBIDO FOR SIGNIFICANT DIFFERENCES

GROUPS COMPARED				STATISTICAL SIGNIFICANCE
GROUP NO.	OVERALL INCIDENCE OF POOR LIBIDO	GROUP NO.	OVERALL INCIDENCE OF POOR LIBIDO	P <
1	0	2	71.4	0.001
1	0	3	38.5	0.001
1	0	4	44.4	0.001
1	0	5	80.0	0.001
1	0	6	25.0	0.001
1	0	7	33.3	0.001
2	71.4	3	38.5	0.01
2	71.4	4	44.4	0.05
2	71.4	5	80.0	..
2	71.4	6	25.0	0.01
2	71.4	7	33.3	0.01
3	38.5	4	44.4	..
3	38.5	5	80.0	0.01
3	38.5	6	25.0	..
3	38.5	7	33.3	..
4	44.4	5	80.0	0.05
4	44.4	6	25.0	..
4	44.4	7	33.3	..
5	80.0	6	25.0	0.01
5	80.0	7	33.3	0.01
6	25.0	7	33.3	..
8 A	52.0	8 B	48.0	..
8 A	52.0	8 C	48.0	..
8 A	52.0	8 D	54.0	..
8 A	52.0	8 E	55.1	..
8 B	48.0	8 C	48.0	..
8 B	48.0	8 D	54.0	..
8 B	48.0	8 E	55.1	..
8 C	48.0	8 D	54.0	..
8 C	48.0	8 E	55.1	..
8 D	54.0	8 E	55.1	..

NOTE: 1. See opposite page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

4.1.11 OTHER SYMPTOMS

4.1.11.1 VAGINAL BLEEDING

This symptom was not analysed in this thesis for the reason that no patients were accepted to the study with postmenopausal bleeding and all patients receiving oestrogen therapy had undergone hysterectomy. Thus, although included on the original trial form, the heading in fact was redundant.

4.1.11.2 PERSPIRATION

The incidence of perspiration was the same as that of hot flushes. The figures are not presented in order to prevent repetition. All comments with regard to the symptom of hot flushes apply similarly to the symptom of perspiration.

4.1.11.3 MOOD CHANGES

The assessment of mood change in a large group of patients each seen over a period of one year was extremely difficult and open to too great a margin of error and personal bias. This factor was therefore excluded from the study.

4.1.11.4 PRURITUS VULVAE AND DYSPAREUNIA

Dyspareunia is considered under Section 4.1.2 Atrophic Vaginitis.

The incidence of pruritus vulvae in all groups of patients studied was insignificant and no comment on this symptom is possible.

4.1.12 OVERALL IMPRESSION OF RESPONSE TO TREATMENT

The score on which this assessment is based is defined and discussed under Section 3.2.2.

The overall clinical impression as to the general response of the oophorectomized female to oestrogen and placebo therapy is indicated in Table 29, together with the chi square distribution for statistical significance. Further statistical comparison between the groups on the various forms of treatment is tabulated in Table 31.

The statistical evaluation in this instance requires some qualification. The score of assessment was partially non-parametric; however the results, as shown below, demonstrate striking trends which are of such a degree that, on the advice of a statistician, non-parametric tests of significance were considered unnecessary. The statistical evaluation is thus presented for the sake of completeness.

The patient's personal impression as to her own overall response to treatment, in this instance oestrogen or placebo in the form of a single blind cross-over trial, is shown in Table 30. This table also indicates the chi square distribution for statistical significance. The groups receiving the various forms of treatment are further individually compared to each other for statistical differences and the results listed in Table 32.

Thus Tables 29 and 31 indicate the investigator's impression and Tables 30 and 32 indicate the patient's impression, i.e. Table 29 is analagous to Table 30 and Table 31 to Table 32. Comparison of these two analagous groups of tables show the results of the statistical tests in each instance to be identical. The presentation of the results

below takes this fact into account.

Oestrogen and placebo therapy are both demonstrated to have a highly significant 'tonic effect' on the patient and this is borne out by both the observer's assessment and the patient's impression ($p < 0.001$). That this is not purely a psychological response to an interested physician is shown statistically (Tables 31 and 32) and confirmed by the following finding of significance. Comparison of the oestrogen-treated groups (Groups 8B, 8C and 8E) to the groups of the same patients after single blind cross-over to placebo (Group 8D) indicates that there was a significant deterioration in the patient's overall feeling of well-being whilst on placebo ($p < 0.01$). Nevertheless, a large proportion of patients were unaware of the change from oestrogen to placebo and comparison of the control and placebo groups indicates a statistically highly significant difference ($p < 0.001$).

The possible interpretation of these results is discussed under Part 5.1

TABLE 29

INVESTIGATOR'S OVERALL IMPRESSION OF THE GENERAL RESPONSE OF OOPHORECTOMIZED FEMALES TO OESTROGEN AND PLACEBO THERAPY

	CONTROL		3 MONTHS OESTRADIOL VALERATE		6 MONTHS OESTRADIOL VALERATE		3 MONTHS PLACEBO		3 MONTHS CONJUGATED OESTROGENS		VALUE FOR CHI SQUARE	STATISTICAL SIGNIFICANCE P <
	No.	%	No.	%	No.	%	No.	%	No.	%		
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100		
Marked improvement	0	0	14	28.0	16	32.0	8	16.0	16	32.7	35.425	0.001
Slight improvement	0	0	27	54.0	24	48.0	13	26.0	23	46.9	56.535	0.001
Unchanged	50	100	5	10.0	8	16.0	7	14.0	7	14.3	194.258	0.001
Slight deterioration	0	0	4	8.0	2	4.0	18	36.0	3	6.1	76.511	0.001
Marked deterioration	0	0	0	0	0	0	4	8.0	0	0	31.999	0.001

TABLE 30

PATIENTS' PERSONAL IMPRESSION OF THEIR RESPONSE TO TREATMENT (OESTROGEN AND PLACEBO - SINGLE BLIND CROSS OVER)

	CONTROL		3 MONTHS OESTRADIOL VALERATE		6 MONTHS OESTRADIOL VALERATE		3 MONTHS PLACEBO		3 MONTHS CONJUGATED OESTROGENS		VALUE FOR CHI SQUARE	STATISTICAL SIGNIFICANCE P <
	No.	%	No.	%	No.	%	No.	%	No.	%		
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100		
Marked improvement	0	0	15	30.0	15	30.0	11	22.0	18	36.8	34.244	0.001
Slight improvement	0	0	25	50.0	29	58.0	13	26.0	22	44.9	60.228	0.001
Unchanged	50	100	6	12.0	6	12.0	5	10.0	6	12.2	214.157	0.001
Slight deterioration	0	0	4	8.0	0	0	17	34.0	3	6.1	82.587	0.001
Marked deterioration	0	0	0	0	0	0	4	8.0	0	0	31.999	0.001

EXPLANATION OF GROUP NUMBERS DESCRIBED IN TABLE OF STATISTICAL COMPARISON

GROUP NO.	DESCRIPTION OF GROUP OF PATIENTS
8	All patients investigated and treated post-oophorectomy
8 A	Control - variable time < 2 years post-oophorectomy
8 B	3 months continuous oestradiol valerate therapy
8 C	6 months continuous oestradiol valerate therapy
8 D	3 months continuous placebo therapy
8 E	3 months continuous conjugated oestrogen therapy

TABLE 31

TREATED GROUPS STATISTICALLY COMPARED FOR OVERALL IMPROVEMENT ON THERAPY (OBSERVER'S ASSESSMENT)

GROUPS COMPARED				STATISTICAL SIGNIFICANCE
GROUP NO.	OVERALL INCIDENCE	GROUP NO.	OVERALL INCIDENCE	P <
8 A	0	8 B	82.0	0.001
8 A	0	8 C	80.0	0.001
8 A	0	8 D	42.0	0.001
8 A	0	8 E	79.6	0.001
8 B	82.0	8 C	80.0	..
8 B	82.0	8 D	42.0	0.01
8 B	82.0	8 E	79.6	..
8 C	80.0	8 D	42.0	0.01
8 C	80.0	8 E	79.6	..
8 D	42.0	8 E	79.6	0.01

TABLE 32

TREATED GROUPS STATISTICALLY COMPARED FOR OVERALL IMPROVEMENT ON THERAPY (PATIENT'S ASSESSMENT)

GROUPS COMPARED				STATISTICAL SIGNIFICANCE
GROUP NO.	OVERALL INCIDENCE	GROUP NO.	OVERALL INCIDENCE	P <
8 A	0	8 B	80.0	0.001
8 A	0	8 C	88.0	0.001
8 A	0	8 D	48.0	0.001
8 A	0	8 E	81.7	0.001
8 B	80.0	8 C	88.0	..
8 B	80.0	8 D	48.0	0.01
8 B	80.0	8 E	81.7	..
8 C	88.0	8 D	48.0	0.01
8 C	88.0	8 E	81.7	..
8 D	48.0	8 E	81.7	0.01

NOTE: 1. See opposite page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

4.2 ANALYSIS OF SIGNS

4.2.1 BODY WEIGHT

The mean body weight of the patients in the various groups studied is shown in Table 33. This table indicates that the mean body weight in the several groups varied between 122.63 and 162.67. This wide variation is reflected in the standard deviations and standard errors and no purpose is served by comparing these groups statistically.

The group of oophorectomized patients undergoing the trial scheme of therapy, however, could be compared statistically as this was an identical group being compared on different forms of therapy. The effect of oestrogen and placebo therapy on the body weight of these oophorectomized females is shown in Table 34.

The mean body weight, standard deviation and standard error of these groups are remarkably similar and statistically show no difference. It may therefore be concluded that the administration of oestrogens to oophorectomized females does not result in any significant change in the body weight.

TABLE 33

MEAN BODY WEIGHT, STANDARD DEVIATION AND STANDARD ERROR IN POUNDS OF THE GROUPS OF PATIENTS INVESTIGATED

BODY WEIGHT	NORMAL	POST-OOPHORECTOMY				CONSERVED OVARIES	
	PREMENOPAUSAL	PREMENOPAUSAL			POSTMENOPAUSAL	POST-HYSTERECTOMY	
		Immediate	6 months	2 years	Immediate	6 months	2 years
Total No. of patients studied	9	14	13	18	5	8	18
Body Weight (lbs.)							
Mean	155.89	140.29	150.08	152.61	125.80	122.63	162.67
SD	66.95	17.80	32.00	27.99	17.02	55.60	38.52
SE	9.56	4.94	8.88	12.52	7.61	7.94	5.50

TABLE 34

THE EFFECT OF OESTROGEN AND PLACEBO THERAPY ON THE BODY WEIGHT OF OOPHORECTOMIZED FEMALES

BODY WEIGHT	CONTROL	3 MONTH OESTRADIOL VALERATE	6 MONTH OESTRADIOL VALERATE	3 MONTH PLACEBO	3 MONTH CONJUGATED OESTROGENS
No. of Patients	50	50	50	50	49
Mean body weight in pounds	145.82	146.90	144.94	144.76	146.86
Standard deviation	26.49	25.03	24.81	24.44	25.03
Standard error	3.78	3.58	3.55	3.49	3.58

4.2.2. DIASTOLIC BLOOD PRESSURE

The mean diastolic blood pressure of the various groups investigated is shown in Table 35. These figures are presented for the sake of completeness and to indicate that none of the patients admitted to the study was suffering from any major degree of hypertension.

The effect of oestrogen and placebo therapy on the diastolic blood pressure of oophorectomized females is indicated in Table 36. No statistical differences of significance were demonstrated between any of the groups. It is concluded that oestrogen therapy does not cause a rise in the diastolic blood pressure.

TABLE 35

AVERAGE DIASTOLIC BLOOD PRESSURE (MM. HG.) OF THE GROUPS OF PATIENTS INVESTIGATED

DIASTOLIC BLOOD PRESSURE	NORMAL		POST-OOPHORECTOMY								CONSERVED OVARIES			
	PREMENOPAUSAL		PREMENOPAUSAL						POSTMENOPAUSAL		POST-HYSTERECTOMY			
			Immediate		6 months		2 years		Immediate		6 months		2 years	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients studied	9	100	14	100	13	100	18	100	5	100	8	100	18	100
Diastolic														
< 90	7	77.8	9	64.3	6	46.2	9	50.0	4	80.0	5	62.5	10	55.6
90 - 120	2	22.2	5	35.7	7	53.8	9	50.0	1	20.0	3	37.5	7	38.9
> 120	0	0	0	0	0	0	0	0	0	0	0	0	1	5.5

TABLE 36

THE DIASTOLIC BLOOD PRESSURE (MM. HG.) OF OOPHORECTOMIZED FEMALES AND THE EFFECT OF OESTROGEN AND PLACEBO THERAPY

DIASTOLIC BLOOD PRESSURE	CONTROL		3 MONTH OESTRADIOL VALERATE		6 MONTH OESTRADIOL VALERATE		3 MONTH PLACEBO		3 MONTH CONJUGATED OESTROGENS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100
< 90	28	56.0	35	70.0	33	66.0	31	62.0	27	55.1
90 - 120	22	44.0	15	30.0	17	34.0	19	38.0	20	40.8
> 120	0	0	0	0	0	0	0	0	2	4.1

4.2.3 BREASTS

The clinical state of the breasts viz. normal or atrophic in the several groups of patients investigated is listed in Table 37. The effect of oestrogen and placebo therapy on this clinical state in oophorectomized females is considered in Table 38. The statistical comparison for significant differences between the groups of patients investigated and the overall incidence of atrophic breasts is summarised in Table 39.

There is a statistically highly significant difference in the incidence of atrophic breasts in all the oophorectomized groups of patients as compared with the normal premenopausal group of patients ($p < 0.001$).

Comparison of the oophorectomized and non-oophorectomized groups of patients assessed 6 months after surgery indicates a significantly higher incidence of atrophic breasts in the oophorectomized group ($p < 0.01$). This relationship also exists for the 2 year groups, but to a lesser degree of statistical significance ($p < 0.05$).

However, a highly significant difference exists between the normal premenopausal group and that group of patients investigated two years after hysterectomy with conservation of ovaries ($p < 0.001$). This is an unexpected finding.

All patients in the postmenopausal group (100%) assessed immediately after oophorectomy had atrophic breasts and statistical differences were found in comparing this group to the normal premenopausal females ($p < 0.001$); the premenopausal patients assessed immediately post-oophorectomy ($p < 0.01$); the premenopausal patients assessed 6 months after hysterectomy with conservation of ovaries ($p < 0.001$) and the premenopausal

patients assessed 2 years after hysterectomy with conservation of ovaries ($p < 0.01$).

Oestrogen therapy produced no alteration in the clinical state of the breasts of oophorectomized females.

The most logical interpretation of the above results would seem to be that normal ovarian function is necessary for the maintenance of normal breast configuration. Oophorectomy results in atrophy of the breasts. Similarly, decreased ovarian function after the menopause has the same effect. Despite these findings, exogenous oestrogen therapy, of the two types used in the present investigation, has been shown to be of no value in restoring atrophic breasts to a normal premenopausal state.

TABLE 37

CLINICAL STATE OF THE BREASTS IN THE GROUPS OF PATIENTS INVESTIGATED

BREASTS	NORMAL		POST-OOPHORECTOMY								CONSERVED OVARIES			
	PREMENOPAUSAL		PREMENOPAUSAL						POSTMENOPAUSAL		POST-HYSTERECTOMY			
			Immediate		6 months		2 years		Immediate		6 months		2 years	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients studied	9	100	14	100	13	100	18	100	5	100	8	100	18	100
Normal	8	88.9	7	50.0	5	38.5	5	27.8	0	0	6	75.0	9	50.0
Atrophic	1	11.1	7	50.0	8	61.5	13	72.2	5	100	2	25.0	9	50.0

TABLE 38

THE CLINICAL STATE OF THE BREASTS OF OOPHORECTOMIZED FEMALES AND THE EFFECT OF OESTROGEN AND PLACEBO THERAPY

BREASTS	CONTROL		3 MONTH OESTRADIOL VALERATE		6 MONTH OESTRADIOL VALERATE		3 MONTH PLACEBO		3 MONTH CONJUGATED OESTROGENS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100
Normal	17	34.0	19	38.0	16	32.0	13	26.0	17	34.7
Atrophic	33	66.0	31	62.0	34	68.0	37	74.0	32	65.3

EXPLANATION OF GROUP NUMBERS DESCRIBED
IN TABLE OF STATISTICAL COMPARISON

GROUP NO.	DESCRIPTION OF GROUP OF PATIENTS
1	Normal premenopausal
2	Premenopausal, investigated immediately post-oophorectomy
3	Premenopausal, investigated 6 months post-oophorectomy
4	Premenopausal, investigated 2 years post-oophorectomy
5	Postmenopausal, investigated immediately post-oophorectomy
6	6 months post-hysterectomy with conserved ovaries
7	2 years post-hysterectomy with conserved ovaries
8	All patients investigated and treated post-oophorectomy (i.e. Groups 2+3+4+5)
8 A	Control - variable time < 2 years post-oophorectomy
8 B	3 months continuous oestradiol valerate therapy
8 C	6 months continuous oestradiol valerate therapy
8 D	3 months continuous placebo therapy
8 E	3 months continuous conjugated oestrogen therapy

TABLE 39

STATISTICAL COMPARISON BETWEEN THE GROUPS OF PATIENTS INVESTIGATED AND THE OVERALL INCIDENCE OF ATROPHIC BREASTS

GROUPS COMPARED				STATISTICAL SIGNIFICANCE
GROUP NO.	OVERALL INCIDENCE OF ATROPHIC BREASTS	GROUP NO.	OVERALL INCIDENCE OF ATROPHIC BREASTS	P <
1	11.1	2	50.0	0.001
1	11.1	3	61.5	0.001
1	11.1	4	72.2	0.001
1	11.1	5	100.0	0.001
1	11.1	6	25.0	..
1	11.1	7	50.0	0.001
2	50.0	3	61.5	..
2	50.0	4	72.2	..
2	50.0	5	100.0	0.01
2	50.0	6	25.0	0.05
2	50.0	7	50.0	..
3	61.5	4	72.2	..
3	61.5	5	100.0	0.05
3	61.5	6	25.0	0.01
3	61.5	7	50.0	..
4	72.2	5	100.0	0.05
4	72.2	6	25.0	0.01
4	72.2	7	50.0	0.05
5	100.0	6	25.0	0.001
5	100.0	7	50.0	0.01
6	25.0	7	50.0	0.05
8 A	66.0	8 B	62.0	..
8 A	66.0	8 C	68.0	..
8 A	66.0	8 D	74.0	..
8 A	66.0	8 E	65.3	..
8 B	62.0	8 C	68.0	..
8 B	62.0	8 D	74.0	..
8 B	62.0	8 E	65.3	..
8 C	68.0	8 D	74.0	..
8 C	68.0	8 E	65.3	..
8 D	74.0	8 E	65.3	..

NOTE: 1. See opposite page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

4.2.4. OTHER SIGNS

4.2.4.1 HAIR TEXTURE

Hair texture proved to be an extremely difficult sign in which to determine change. It is therefore not possible to present any meaningful figures. Nevertheless, after assessment of the patient's response to history (any gain or loss of hair, change in quality, etc.) and the author's personal impression, the conclusion arrived at is that both oophorectomy and subsequent exogenous oestrogen therapy appear to have little effect on the quality or texture of scalp hair.

4.2.4.2 SKIN THICKNESS

Skin thickness results are not presented because skin-fold measurements subsequently proved to be inaccurate in the obese patient. Exclusion of these patients from the sub-groups investigated reduced the size of the groups so that statistical evaluation would have been meaningless. It was therefore not possible to arrive at any conclusion with regard to skin thickness.

4.2.4.3 HEIGHT

No change in body height was demonstrated in the oophorectomized group of patients receiving oestrogen therapy. No purpose is therefore served by presenting these figures and they have been excluded from the study.

4.3 VAGINAL SMEAR

The details of the vaginal epithelial cell counts of all patients investigated in this study appear in Appendix B (Tables B.1 and B.2) as Maturation Indices (parabasal : intermediate : superficial cell types). The vaginal epithelial cell types, with mean, standard deviation , standard error and statistical significance in the several groups of patients investigated, are shown in Table 40. The effect of oestrogen and placebo therapy on the vaginal epithelial cell types (mean, standard deviation, standard error and statistical significance) of oophorectomized females is summarised in Table 41. These changes are diagrammatically represented in Figure 2.

The mean maturation indices of the normal premenopausal, 6 month-post-hysterectomy-conserved-ovaries and 2 year post-hysterectomy-conserved-ovaries groups were 1·22 : 89·00 : 9·78, 0·00 : 92·87 : 7·13 and 2·44 : 91·33 : 6·23 respectively. These all indicate an absent or low parabasal cell count with the majority of cells being of intermediate type. The superficial cell counts are relatively low.

The mean parabasal cell counts in the oophorectomized groups range from 4·69 to 15·22; mean intermediate cell counts from 79·93 to 84·23 and mean superficial cell counts from 3·33 to 11·08.

The statistical comparison between the groups with intact ovaries against those without ovaries indicates a highly significant difference for the parabasal cell counts ($p < 0.005$), but not for the intermediate or superficial cell counts.

Oestrogen therapy is shown to promote the maturation of vaginal epithelial cells from the parabasal to the

intermediate type (Table 41 and Figure 2). Numerically, however, the only cell type to reflect a significant change is the parabasal. The mean parabasal control value of 11·54 is reduced to 0·54 after 3 months' continuous oestradiol valerate therapy and is 1·12 at 6 months. Single blind cross-over to placebo results in a significant rise of the mean value to 8·34. The final cross-over of placebo to conjugated oestrogen results in a drop of the mean value, once again, to 0·71. These results are statistically highly significant ($p < 0.0005$). The mean intermediate cell count ranged between a control value of 81·70 and 93·29 after 3 months' conjugated oestrogen therapy. This difference is not statistically significant.

The superficial cell count varied between mean values of 2·16 and 8·04 (Table 41), the former following placebo therapy and the latter after 6 months' continuous oestradiol valerate therapy. The fact that continuous high dosage oestrogen therapy was unable to raise the superficial cell index beyond a mean of 8·04 is considered to be a significant finding.

There was no statistical difference between the two types of oestrogen used in their effect on the vaginal epithelium.

In summary, therefore, the above results demonstrate that oophorectomy produces a statistically significant increase in the vaginal smear parabasal cell count and this response is reversed by the administration of exogenous oestrogens ($p < 0.0005$). No significant changes in the intermediate or superficial cell counts result from either oophorectomy or oestrogen administration.

TABLE 40

VAGINAL EPITHELIAL CELL POPULATION EXPRESSED AS PERCENTILES (MEAN, STANDARD DEVIATION AND STANDARD ERROR) OF THE GROUPS OF PATIENTS STUDIED

	NORMAL PREMENOPAUSAL	POST-OOPHORECTOMY				CONSERVED OVARIES POST-HYSTERECTOMY		STATISTICAL SIGNIFICANCE P <
		PREMENOPAUSAL			POSTMENOP.			
		Immediate	6 months	2 years	Immediate	6 months	2 years	
Total No. of patients studied	9	14	13	18	5	8	18	
PARABASAL MEAN	1.22	12.36	4.69	15.22	13.80	0.00	2.44	Highly Significant 0.0005
S.D.	3.31	22.06	7.15	22.05	17.08	0.00	5.64	
S.E.	0.47	6.12	1.98	9.86	7.64	0.00	0.81	
INTERMEDIATE MEAN	89.00	79.93	84.23	81.44	81.00	92.87	91.33	Not Significant 0.95
S.D.	10.97	20.78	16.50	20.04	15.70	7.16	12.17	
S.E.	1.57	5.77	4.58	8.96	7.02	1.02	1.74	
SUPERFICIAL MEAN	9.78	7.71	11.08	3.33	5.20	7.13	6.23	Not Significant 0.50
S.D.	11.12	8.71	17.57	4.77	9.44	7.16	11.65	
S.E.	1.59	2.42	4.87	2.13	4.23	1.02	1.67	

TABLE 41

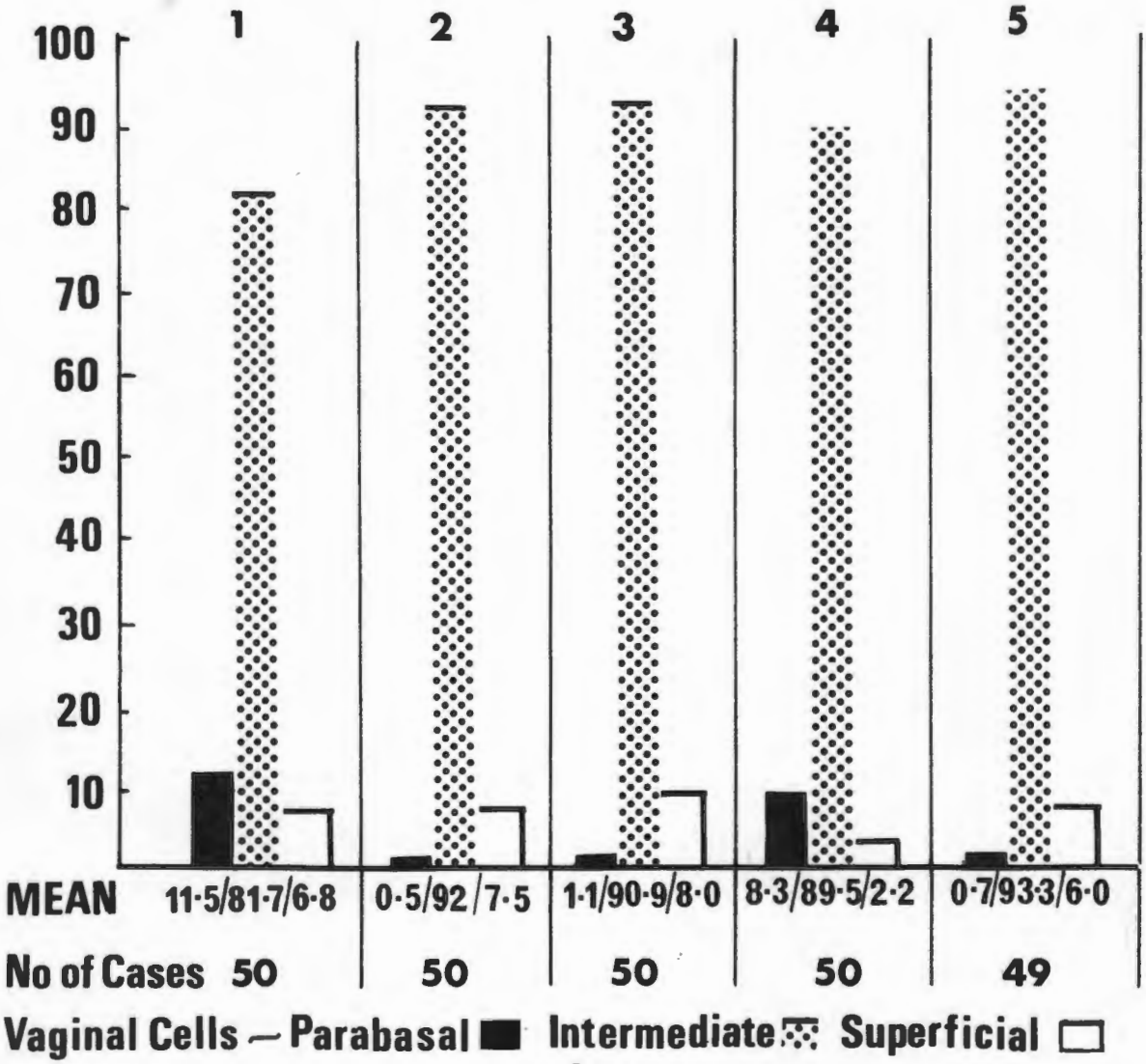
MEAN RESPONSE WITH STANDARD DEVIATION, STANDARD ERROR AND SIGNIFICANCE OF VAGINAL EPITHELIAL CELLS TO OESTROGEN AND PLACEBO THERAPY

	CONTROL	3 MONTH OESTRADIOL VALERATE	6 MONTH OESTRADIOL VALERATE	3 MONTH PLACEBO	3 MONTH CONJUGATED OESTROGENS	STATISTICAL SIGNIFICANCE
Total No. of patients treated	50	50	50	50	49	
PARABASAL MEAN	11.54	0.54	1.12	8.34	0.71	Highly
S.D.	18.77	1.40	4.56	17.18	1.32	Significant
S.E.	2.68	0.20	0.65	2.45	0.19	$p < 0.0005$
INTERMEDIATE MEAN	81.70	92.00	90.92	89.50	93.29	Not
S.D.	18.53	8.55	11.51	16.88	9.70	Significant
S.E.	2.65	1.22	1.65	2.41	1.39	$p < 0.95$
SUPERFICIAL MEAN	6.76	7.46	8.04	2.16	6.00	Not
S.D.	10.99	8.83	11.35	4.10	9.94	Significant
S.E.	1.57	1.26	1.62	0.59	1.42	$p < 0.50$

FIGURE 2

DIAGRAMMATIC REPRESENTATION OF CHANGE IN MATURATION INDEX INDUCED BY OESTROGEN AND PLACEBO THERAPY.

VISIT 1 = CONTROL; 2 = 3 MONTHS OESTRADIOL VALERATE; 3 = 6 MONTHS OESTRADIOL VALERATE; 4 = PLACEBO; 5 = 3 MONTHS CONJUGATED OESTROGEN THERAPY.



4.4 SERUM CHOLESTEROL

The serum cholesterol values of all patients investigated in this study appear in Appendix C (Tables C.1 and C.2). The mean values in mg per 100 ml, with standard deviation and standard error, of the several groups investigated appear in Table 42 and the response to oestrogen and placebo therapy is indicated in Table 43. The statistical comparison between these groups for significant differences in serum cholesterol values appears in Table 44.

The mean serum cholesterol of the several groups investigated ranged from 241.60 to 268.56 (S.D.:30.23 to 54.82; S.E.: 4.32 to 20.95).

There is considerable overlap in these values and no significant statistical differences exist between the various groups investigated (Table 44). For example, oophorectomy is not shown to increase significantly the serum cholesterol value. Thus no differences are found by comparing the 6 month oophorectomized group mean value of 250.85 with the 6 month conserved-ovary group mean value of 242.00; nor by comparing the mean values of 265.44 and 260.39 of the 2 year groups without and with ovaries respectively.

Oestradiol valerate therapy to oophorectomized females reduced the mean control value from 255.08 mg per 100 ml to 244.44 at 3 months and 242.04 at 6 months. Cross-over to placebo resulted in an increase to 259.72 mg per 100 ml. This effect of oestradiol valerate on the reduction of serum cholesterol value was of possible significance ($p < 0.05$). Conjugated oestrogen therapy, however, had no significant effect.

Thus, the present investigation has not shown oophorectomy to result in a significant increase in the serum cholesterol

value. Nevertheless, oestrogen therapy in the form of oestradiol valerate was of benefit in reducing the value of the serum cholesterol.

TABLE 42

BLOOD CHOLESTEROL ESTIMATIONS IN MG PER 100 ML (Mean, Standard Deviation and Standard Error) OF THE GROUPS OF PATIENTS STUDIED

BLOOD CHOLESTEROL	NORMAL	POST-OOPHORECTOMY				CONSERVED OVARIES	
	PREMENOPAUSAL	PREMENOPAUSAL			POSTMENOPAUSAL	POST-HYSTERECTOMY	
		Immediate	6 months	2 years	Immediate	6 months	2 years
Total No. of patients studied	9	14	13	18	5	8	18
Mean	268.56	250.50	250.85	265.44	241.60	242.00	260.39
S.D.	43.63	54.82	31.81	41.56	46.84	30.23	41.38
S.E.	6.23	15.20	8.82	18.59	20.95	4.32	5.91

TABLE 43

RESPONSE OF BLOOD CHOLESTEROL (MEAN, STANDARD DEVIATION AND STANDARD ERROR IN MG. PER 100 ML) OF OOPHORECTOMIZED PATIENTS TO OESTROGEN AND PLACEBO THERAPY

BLOOD CHOLESTEROL	CONTROL	3 MONTH OESTRADIOL VALERATE	6 MONTH OESTRADIOL VALERATE	3 MONTH PLACEBO	3 MONTH CONJUGATED OESTROGENS
Total No. of patients treated	50	50	50	50	49
Mean	255.08	244.44	242.04	259.72	253.94
S.D.	43.50	37.42	34.40	48.60	41.92
S.E.	6.21	5.35	4.92	6.94	5.99

EXPLANATION OF GROUP NUMBERS DESCRIBED IN TABLE OF STATISTICAL COMPARISON

GROUP NO.	DESCRIPTION OF GROUP OF PATIENTS
1	Normal premenopausal
2	Premenopausal, investigated immediately post-oophorectomy
3	Premenopausal, investigated 6 months post-oophorectomy
4	Premenopausal, investigated 2 years post-oophorectomy
5	Postmenopausal, investigated immediately post-oophorectomy
6	6 months post-hysterectomy with conserved ovaries
7	2 years post-hysterectomy with conserved ovaries
8	All patients investigated and treated post-oophorectomy (i.e. Groups 2+3+4+5)
8 A	Control - variable time $<$ 2 years post-oophorectomy
8 B	3 months continuous oestradiol valerate therapy
8 C	6 months continuous oestradiol valerate therapy
8 D	3 months continuous placebo therapy
8 E	3 months continuous conjugated oestrogen therapy

TABLE 44

STATISTICAL COMPARISON BETWEEN THE GROUPS OF PATIENTS INVESTIGATED FOR SIGNIFICANT DIFFERENCES IN BLOOD CHOLESTEROL VALUES

GROUPS COMPARED		STATISTICAL SIGNIFICANCE			
GROUP NO.	TO GROUP NO.	DEGREES FREEDOM	T VALUE	P =<	2P =<
1	2	21	0.8311	..	..
1	3	20	1.1040	..	..
1	4	25	0.1804	..	..
1	5	12	1.4495	..	..
1	6	15	1.4394	..	..
1	7	25	0.4749	..	..
2	3	25	0.0197	..	..
2	4	30	0.8780	..	..
2	5	17	0.9937	..	..
3	6	19	0.6302	..	..
4	7	34	0.6670	..	..
6	7	24	1.5554	..	..
8 A	8 B	98	1.3112	..	..
8 A	8 C	98	1.6626	0.05	0.10
8 A	8 D	98	0.5031	..	..
8 A	8 E	97	0.1328	..	..
8 B	8 D	98	1.7615	0.05	0.10
8 B	8 E	97	1.1899	..	..
8 C	8 D	98	2.0996	0.025	0.05
8 D	8 E	97	0.6332	..	..

NOTE: 1. See opposite page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

4.5 CALCIUM, PHOSPHORUS AND ALKALINE PHOSPHATASE

Plasma calcium, inorganic phosphorus and alkaline phosphatase values of all patients investigated in this study appear in Appendix D (Tables D.1, D.2, D.3 and D.4). Mean plasma calcium (in mg per 100 ml) inorganic phosphorus (in mg per 100 ml) and alkaline phosphatase values (in Bodansky-Reinhart units) of the groups of patients studied appear in Table 45. The response of plasma calcium, inorganic phosphorus and alkaline phosphatase values (all with mean, Standard Deviation - S.D. and Standard Error - S.E.) of oophorectomized patients following oestrogen and placebo therapy is summarised in Table 46. The statistical comparisons between the groups of patients investigated, for significant differences in plasma calcium and inorganic phosphorus, appear in Tables 47 and 48 respectively.

There was a range for plasma calcium in the groups studied of 9·48 to 9·88 mg per 100 ml (Table 45). The statistical comparison between these groups showed no significant differences (Table 47). Hence comparison between normal premenopausal patients and groups following hysterectomy with and without ovarian conservation showed no difference in the range of values of plasma calcium. Nevertheless, administration of conjugated equine oestrogen resulted in a significant lowering of the mean plasma calcium value to 9·24 from a control value of 9·68 ($p < 0.0025$) and a placebo value of 9·55 ($p < 0.01$). In this effect conjugated oestrogen was also shown to be statistically superior to oestradiol valerate ($p < 0.0125$).

The plasma inorganic phosphorus results were more difficult to interpret (Tables 45 and 48). The mean range for plasma inorganic phosphorus values in the groups studied was 2·99

to 3·93. Excluding the 2 year-post-hysterectomy-conserved-ovaries group (Group 7), this range was 3·46 to 3·93. The mean value of 2·99 in Group 7 was thus strikingly low and showed statistical differences to the normal premenopausal ($p < 0.005$) 2 year-post-oophorectomy ($p < 0.0005$) and 6 month-retained-ovaries ($p < 0.005$) groups. The mean normal premenopausal value was 3·61. The immediate post-oophorectomy value of 3·93 was higher but not significantly different, although this value fell to 3·53 at 6 months post-oophorectomy ($p < 0.05$) and to 3·46 at 2 years ($p < 0.01$). Neither of these latter two values differed significantly from the normal premenopausal value. Comparison of the mean values of 3·53 and 3·57 for the 6 month oophorectomized and conserved-ovaries groups respectively showed no statistical significance.

Although these findings suggest that the ovary is effective in either producing a reduction in or preventing an elevation of plasma inorganic phosphorus, it is nevertheless impossible to place any strict interpretation on these results.

The effect of exogenous oestrogen replacement therapy on plasma inorganic phosphorus values in the oophorectomized female is thus considered to be of importance. In fact, such therapy is shown to be effective in reducing the level of plasma inorganic phosphorus (Tables 46 and 48). Both forms of oestrogen used in this study were equally effective. Oestradiol valerate therapy for 3 months resulted in a reduction of the mean value from 3·63 to 3·39 ($p < 0.025$). Assessment after 6 months of oestradiol valerate showed a mean value of 3·49 and this was not significantly different from the original mean control value. Re-conversion to placebo resulted in an increase of the mean value to 3·62, a value similar to that of the original control group. The subsequent cross-over to

conjugated oestrogen resulted in a statistically highly significant drop of the mean values from 3.62 to 3.24 ($p < 0.0025$) and comparison of this latter value with the original mean control value was also statistically highly significant ($p < 0.0005$).

The average values for alkaline phosphatase in all the groups investigated were well within the range of normality (2.52 to 4.58 Bodansky-Reinhart Units). This is supportive evidence that no patients admitted to the investigation were suffering from any manifest form of bone disease. The mean values with S.D. and S.E. are presented for the sake of completeness in Tables 45 and 46.

TABLE 45

PLASMA CALCIUM (MG per 100 ML) INORGANIC PHOSPHORUS (MG per 100 ML) AND ALKALINE PHOSPHATASE (BODANSKY-REINHART UNITS) VALUES OF THE GROUPS OF PATIENTS STUDIED

	NORMAL	POST-OOPHORECTOMY				CONSERVED OVARIES	
	PREMENOPAUSAL	PREMENOPAUSAL			POSTMENOPAUSAL	POST-HYSTERECTOMY	
		Immediate	6 months	2 years	Immediate	6 months	2 years
Total No. of patients studied	9	14	13	18	5	8	18
Calcium - Mean	9.66	9.64	9.48	9.80	9.88	9.76	9.64
S.D.	0.56	0.41	0.92	0.70	0.19	0.36	0.46
S.E.	0.08	0.12	0.26	0.32	0.09	0.05	0.07
Phosphorus - Mean	3.61	3.93	3.53	3.46	3.66	3.57	2.99
S.D.	0.49	0.55	0.50	0.44	0.42	0.86	0.51
S.E.	0.07	0.15	0.14	0.20	0.19	0.12	0.07
Alkaline Phos. Mean	3.00	2.99	3.47	3.33	4.58	2.52	2.84
S.D.	0.95	1.11	0.81	1.45	2.12	1.22	1.76
S.E.	0.14	0.31	0.23	0.65	0.95	0.17	0.25

TABLE 46

RESPONSE OF PLASMA CALCIUM (MEAN, STANDARD DEVIATION AND STANDARD ERROR IN MG PER 100 ML) PHOSPHORUS (MEAN, S.D. AND S.E. IN MG PER 100 ML) AND ALKALINE PHOSPHATASE (MEAN, S.D. AND S.E. IN BODANSKY-REINHART UNITS) VALUES OF OOPHORECTOMIZED PATIENTS TO OESTROGEN AND PLACEBO THERAPY

		CONTROL	3 MONTH OESTRADIOL VALERATE	6 MONTH OESTRADIOL VALERATE	3 MONTH PLACEBO	3 MONTH CONJUGATED OESTROGENS
Total No. of patients treated		50	50	50	50	49
CALCIUM	Mean	9.68	9.54	9.59	9.55	9.24
	S.D.	0.67	0.61	0.64	0.54	0.68
	S.E.	0.10	0.09	0.09	0.08	0.10
PHOSPHORUS	Mean	3.63	3.39	3.49	3.62	3.24
	S.D.	0.51	0.64	0.67	0.63	0.61
	S.E.	0.07	0.09	0.10	0.09	0.09
ALKALINE PHOSPHATASE	Mean	3.40	2.56	2.95	3.28	3.17
	S.D.	1.33	1.45	1.19	1.17	1.29
	S.E.	0.19	0.21	0.17	0.17	0.19

EXPLANATION OF GROUP NUMBERS DESCRIBED IN TABLE OF STATISTICAL COMPARISON

GROUP NO.	DESCRIPTION OF GROUP OF PATIENTS
1	Normal premenopausal
2	Premenopausal, investigated immediately post-oophorectomy
3	Premenopausal, investigated 6 months post-oophorectomy
4	Premenopausal, investigated 2 years post-oophorectomy
5	Postmenopausal, investigated immediately post-oophorectomy
6	6 months post-hysterectomy with conserved ovaries
7	2 years post-hysterectomy with conserved ovaries
8	All patients investigated and treated post-oophorectomy (i.e. Groups 2+3+4+5)
8 A	Control - variable time $<$ 2 years post-oophorectomy
8 B	3 months continuous oestradiol valerate therapy
8 C	6 months continuous oestradiol valerate therapy
8 D	3 months continuous placebo therapy
8 E	3 months continuous conjugated oestrogen therapy

TABLE 47

STATISTICAL COMPARISON BETWEEN THE GROUPS OF PATIENTS INVESTIGATED FOR SIGNIFICANT DIFFERENCES IN PLASMA CALCIUM VALUES

GROUPS COMPARED		STATISTICAL SIGNIFICANCE			
GROUP NO.	TO GROUP NO.	DEGREES FREEDOM	T VALUE	P =<	2P =<
1	2	21	0.0975	..	..
1	3	20	0.4939	..	..
1	4	25	0.5362	..	..
1	5	12	0.7423	..	..
1	6	15	0.4595	..	..
1	7	25	0.0547	..	..
2	3	25	0.5568	..	..
2	4	30	0.7773	..	..
3	6	19	0.8092	..	..
4	7	34	1.3181	..	..
6	7	24	0.4723	..	..
8 A	8 B	98	1.0759	..	..
8 A	8 C	98	0.6589	..	..
8 A	8 D	98	1.1000	..	..
8 A	8 E	97	3.2093	0.0025	0.005
8 B	8 D	98	0.0304	..	..
8 B	8 E	97	2.2822	0.0125	0.025
8 C	8 D	98	0.4054	..	..
8 D	8 E	97	2.4343	0.01	0.02

NOTE: 1. See opposite page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

TABLE 48

STATISTICAL COMPARISON BETWEEN THE GROUPS OF PATIENTS INVESTIGATED FOR SIGNIFICANT DIFFERENCES IN PLASMA INORGANIC PHOSPHORUS VALUES

GROUPS COMPARED		STATISTICAL SIGNIFICANCE			
GROUP NO.	TO GROUP NO.	DEGREES FREEDOM	T VALUE	P =<	2P =<
1	2	21	1.4166	..	..
1	3	20	0.3729	..	..
1	4	25	0.8016	..	..
1	5	12	0.1099	..	..
1	6	15	0.1084	..	..
1	7	25	3.0441	0.005	0.01
2	3	25	1.9664	0.05	0.10
2	4	30	2.6756	0.01	0.02
3	4	29	0.8203	..	..
3	6	19	0.1501	..	..
4	7	34	4.0894	0.0005	0.001
6	7	24	2.9881	0.005	0.01
8 A	8 B	98	2.080	0.025	0.05
8 A	8 C	98	1.1714	..	..
8 A	8 D	98	0.087	..	..
8 A	8 E	97	3.4638	0.0005	0.001
8 B	8 D	98	1.8205	0.05	0.10
8 B	8 E	97	1.2086	..	..
8 C	8 D	98	0.9990	..	..
8 D	8 E	97	3.0658	0.0025	0.005

NOTE: 1. See previous page for explanatory code of group numbers

2. The symbol .. means statistically insignificant

4.6 COMPLICATIONS OF THERAPY

4.6.1 NAUSEA AND VOMITING

The incidence of nausea and vomiting shown in Table 49 was statistically significant after 3 months of oestrogen therapy, irrespective of the type used ($p < 0.01$), the respective incidences being 12% with oestradiol valerate and 18.4% with conjugated equine oestrogens. The incidence decreased to 4% after 6 months of oestradiol valerate therapy, a figure similar to that of the placebo group.

Nausea is thus demonstrated to be a side effect of oestrogen therapy although the high incidence found is most probably related to the high dosage of hormone administered during this trial.

4.6.2 FACIAL HAIR

Seventeen (34 per cent) of the 50 oophorectomized patients exhibited some degree of facial hirsuties as determined both by clinical examination and photographic evidence (face and profile) at the first (control) visit. No further patients developed hirsuties during the programme of investigation.

4.6.3 ALLERGIC MANIFESTATIONS

No allergic manifestations to the hormones administered occurred during the investigation.

4.6.4 ACNE

No cases of acne resulted from either of the types of oestrogen administered.

TABLE 49

THE INCIDENCE OF NAUSEA AND VOMITING IN OOPHORECTOMIZED FEMALES ON OESTROGEN AND PLACEBO THERAPY

	CONTROL		3 MONTHS OESTRADIOL VALERATE		6 MONTHS OESTRADIOL VALERATE		3 MONTHS PLACEBO		3 MONTHS CONJUGATED OESTROGENS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total No. of patients treated	50	100	50	100	50	100	50	100	49	100
Nausea and Vomiting										
Absent	50	100	44	88.0	48	96.0	48	96.0	40	81.6
Present	0	0	6	12.0	2	4.0	2	4.0	9	18.4

4.6.5 VASCULAR THROMBOSIS

One patient presented with superficial thrombophlebitis of a varicose vein 4 weeks after commencing conjugated equine oestrogen therapy. This responded completely to supportive therapy and the oestrogen therapy was not discontinued at any stage. It is not possible to comment on whether this case was a complication of oestrogen therapy or a purely coincidental event.

No cases of deep vein thrombosis, pulmonary embolus, myocardial infarction or cerebral infarction occurred during the study.

PART 5

DISCUSSION

5. DISCUSSION

5.1 CLINICAL FEATURES OF POSTMENOPAUSE, OOPHORECTOMY AND OESTROGEN THERAPY

The lack of general agreement as to the precise symptomatology of the climacteric is reviewed in Part 1.3. It is stressed that there is no scientific evidence differentiating those symptoms related to oestrogen withdrawal from the broad group of symptoms which may be due to the concurrent ageing process or general environmental problems.

The results of the present investigation would thus appear to be of value in that a wide spectrum of symptomatology has been analysed and those symptoms related directly to oestrogen withdrawal following bilateral oophorectomy have been defined. Moreover, further evidence of oestrogen dependence is presented by differentiating those symptoms responding to exogenous oestrogen replacement therapy from those symptoms responding to placebo therapy.

The symptoms, sone of which are discussed individually below, are shown to fall into 5 (five) possible groups :

1. Symptoms directly related to oophorectomy and significantly relieved by exogenous oestrogen therapy
   - A. Hot flushes and perspiration
   - B. Atrophic vaginitis (pain, dyspareunia or blood-stained discharge)
2. Symptoms directly related to oophorectomy but not relieved by exogenous oestrogen therapy
   - A. Angina pectoris
   - B. Depression

3. Symptoms not related to oophorectomy and possibly relieved by exogenous oestrogen therapy

   A. Headache

   B. Irritability

4. Symptoms significantly relieved by placebo therapy

   A. Angina pectoris

   B. Depression

   C. Irritability

   D. Palpitations

   E. Insomnia

5. Symptoms not related to oophorectomy nor relieved by either exogenous oestrogen or placebo therapy

   A. Low backache

   B. Decreased or absent libido (Frigidity)

Consideration of the results presented (Part 4) and the above grouping of symptomatology leads to the conclusion that the only true symptoms of the climacteric relate to hot flushes and atrophic vaginitis. The only other symptom that could remotely be included in this group is that of headache. In this instance, however, there was no relationship to oophorectomy and the response to conjugated oestrogen therapy was of insufficient significance to so relate this symptom. There was a significant incidence of angina pectoris ($p < 0.01$) and depression ($p < 0.05$) following oophorectomy but both responded significantly to placebo therapy. These symptoms are thus more likely to be psychological responses to the operation of oophorectomy than a result of endogenous oestrogen withdrawal. For the same reason irritability, which does not significantly follow oophorectomy but which is relieved by both oestrogen and placebo therapy, is considered not to be

a true symptom of the climacteric. This symptom, however, will be discussed more fully below.

The conclusion that the only symptoms directly resulting from ovarian oestrogen withdrawal following oophorectomy and, by analogy, the normal climacteric, relate to hot flushes and atrophic vaginitis, confirms the general impression that exists in the medical literature to date (Forman, 1968; Greenblatt, 1963; McCandless, 1964; Newton and Odom, 1964; Rogers, 1956a; Squires and Cannell, 1952; Taylor and Rizkallah, 1967; Young, 1939).

Despite the above general conclusions, further discussion on the results relating to certain of the individual symptoms is necessary.

The low incidence of hot flushes in the group of patients assessed two years after hysterectomy with conservation of ovaries is considered to be a significant finding and an important contribution to the present study (see Part 4.1.1). This finding, taken into account with factors discussed above, is considered sufficient evidence to prove that ovaries conserved at the time of hysterectomy on premenopausal women are functional and able to prevent oestrogen dependent symptoms for at least 2 years after such surgery. Support is lent to this argument by the findings of Beavis et al (1969) in a study published following the completion of the present investigation. The function of ovaries conserved at surgery was monitored for periods of 4 to 6 weeks at intervals for up to 2½ years by weekly urinary oestrogen and pregnanediol measurements. Ovaries which were functioning normally before hysterectomy were found to function completely normally after hysterectomy. These findings add considerable weight to the

arguments that wherever possible ovaries should be totally conserved during such surgery.

Irritability has not been demonstrated to be an effect of oophorectomy and is relieved essentially by placebo therapy ($p < 0.05$) (See Part 4.1.7). Conjugated equine oestrogens appear to produce some relief of this symptom over and above the placebo response ($p < 0.05$). Oestradiol valerate was ineffective in this respect. Very similar results have been shown for the symptom of headache. Dapunt (1967) and Velikay (1968) have claimed relief of irritability and headache by the use of oestradiol valerate; neither author, however, has substantiated his findings with statistical or comparative evidence. The only conclusion in the light of these findings is that irritability and headache are not oestrogen related symptoms; the possible relief following oestrogen therapy is most likely related to the general feeling of well-being produced by oestrogen, which is discussed below.

Angina pectoris, depression, irritability, palpitations and insomnia are all symptoms which have been shown to respond significantly to placebo (general supportive) therapy. Angina pectoris, depression and irritability are discussed above. The response of palpitations to placebo therapy and the similar incidence of this symptom in all groups prior to therapy indicates palpitations to be unrelated to oophorectomy and explains the lack of response to oestrogen therapy (see Part 4.1.4). Insomnia demonstrates an even more striking response to placebo therapy which weighs against the possible relationship with oophorectomy ($p < 0.05$) being, in this instance, of any significance.

Whether the response of these symptoms to placebo warrants

such therapy as a routine clinical procedure is conjectural. Kupperman et al (1959) state that 'placebo therapy in such patients is not a dishonest procedure but a means of treating a patient according to the dictums of our profession'.

The symptom of low backache is more real and disabling than, say, irritability or insomnia. No significant relationships were proven between this symptom and oophorectomy or subsequent oestrogen replacement therapy (see Part 4.1.9). The relationship between low backache, osteoporosis and calcium/phosphorus metabolism has been previously reviewed (see Part 1.4.3). The inability of oestrogen therapy to relieve low backache was considered disappointing, especially since oestrogen therapy is shown to affect significantly calcium and phosphorus metabolism in the present study (see Results, Part 4.5 and Discussion, Part 5,4.) These findings are contrary to the experience of Wallach and Henneman (1959), who showed oestrogens to be of considerable value in the treatment of backache.

The most significant finding in relation to the symptom of decreased or absent libido (frigidity) was the high incidence of this problem in all the groups of patients having undergone surgery, irrespective of whether the ovaries had been conserved or not (see Part 4.1.10). The high incidence of this symptom was not unexpected in the groups assessed shortly after surgery; it was not expected in all groups of patients assessed at periods of up to 2 years after hysterectomy. The interpretation placed on these findings is that the operation of hysterectomy, irrespective of whether the ovaries are removed or not, is associated with a deleterious effect on libido. Moreover, this effect has not been shown to be related to oestrogen deprivation; conservation of ovaries does not protect against this symptom, nor has replacement oestrogen therapy been shown

to be of any value in its treatment. Dapunt (1967) failed to show any beneficial effect of oestradiol valerate on libido.

Oophorectomy was not demonstrated to result in any obvious change in facial features, hirsuties, wrinkling of skin or scalp-hair-line configuration. This statement is based on evidence obtained by comparing photographs of patients at each visit (see Part 3.2.2.) These photographs were remarkably similar for any one patient; unfortunately it was not considered practical to incorporate into the body of this thesis copies of the hundreds of photographs taken during the course of this study. Nor, by the same evidence, did exogenous oestrogen therapy demonstrate any beneficial effect on the appearance of oophorectomized females to whom these hormones were administered. It must be conceded that as the time of investigation after oophorectomy and the duration of therapy were both short a more long-term trial is necessary. Nevertheless, the short term effects are considered disappointing.

The one physical characteristic demonstrating significant relationship to menopause and oophorectomy was that of breast configuration. Menopause and oophorectomy are shown to be associated with a significant degree of breast atrophy ($p < 0.001$) (see Part 4.2.3). The finding of a high incidence of atrophic breasts in the group assessed 2 years after ovarian conservation compared to the normal premenopausal group ($p < 0.001$) is difficult to explain. Furthermore, exogenous oestrogen therapy is shown to be of no value in restoring atrophic breasts to a normal premenopausal state. These factors suggest that oestrogens, both endogenous and exogenous, are not the only substances necessary for the maintainance of normal breast tissue. The present thesis does not allow for conjecture as to what these may be.

Administration of oestrogens to oophorectomized females was shown to produce no significant change in the body weight (Part 4.2.1). Nor did such therapy appear to cause a change in the diastolic blood pressure (Part 4.2.2). This is in contrast to the findings of Borgstrom (1954), who reported that in 52 cases of hypertension a lowered blood pressure followed the administration of oestrogen. Taylor et al (1947), however, in a study of 179 oophorectomized women and 21 women with natural menopause found no increase in the prevalence of hypertension over that occurring in the general population and it has been generally agreed that hypertension at the menopause is not hormonally mediated.

The present thesis furnishes statistical confirmation of the general tonic effect with exogenous oestrogen therapy suggested to exist by several authors (Davis, 1967; Greenhill, 1967; Wilson and Wilson, 1963). The application of and rationale behind a score directed at measuring both the observer's and the patient's impression of overall response to therapy is outlined in Part 3.2.2. The results and statistical evaluation of this scoring system are presented in Part 4.1.12. In summary, oestrogen and placebo therapy are both demonstrated to have a significant effect in improving the patient's overall feeling of well-being and this is borne out by both the observer's assessment and the patient's impression ($p < 0.001$). However, single blind cross-over from oestrogen to placebo resulted in a significant deterioration in the patient's overall feeling of well-being ($p < 0.01$). In this respect both forms of oestrogen used in this trial were effective ($p < 0.01$). The interpretation of these results would suggest that oophorectomized females belong to one of three groups. The first group is oestrogen deficient and hence responds to oestrogen replacement therapy,

irrespective of the type used in the present study. The second group responds to oestrogen or placebo therapy hence any beneficial effect is most likely psychological in origin and not in fact drug related at all. The third group is differentiated by its resistance to therapy, both oestrogen and placebo. The most likely explanation in this instance is that a general feeling of poor health is so firmly entrenched psychologically that it cannot be relieved by single placebo therapy. Fortunately this latter group was small, less than 20% of patients showing no beneficial response at all to therapy. The possible explanation for this tonic effect has been theorised by Kupperman et al (1959). Kantor et al (1968) have presented a preliminary report of an investigation still in progress suggesting that oestrogen therapy to ageing women tends to prevent deterioration in psychological behaviour.

The only significant complication or side-effect of oestrogen therapy demonstrated in this study was a high incidence of nausea and vomiting (see Part 4.6.1). This effect was common to both forms of oestrogen administered and is considered to be related to the high dosage of hormone used for the purposes of this trial. One patient developed a superficial thrombophlebitis of a varicose vein 4 weeks after commencing conjugated equine oestrogen therapy (see Part 4.6.5). The literature relating oestrogens with vascular thrombosis is discussed in Part 1.6 (Daniel et al, 1967; Jeffcoate et al, 1968; Mozes et al, 1965; Poller and Thomson, 1966; Poller et al, 1969; Swyer, 1966; Vessey and Doll, 1968, 1969). Although this patient may, in fact, represent such a complication, conclusions about the frequency of such complications can only be derived from large series of patients. Nevertheless, the possibility of oestrogen therapy causing thrombosis appears to be real and the clarification of this problem is considered to be a matter or urgency.

5.2 VAGINAL SMEAR

The literature on vaginal smear has been reviewed in Part 1.4.4. It has been shown that the vaginal smear is a bioassay. A positive correlation has been proven between karyopyknotic index (KI) and urinary output of oestrone, oestradiol, oestriol and total oestrogens in healthy postmenopausal patients (Grönroos, 1965; Procopé, 1968).

The overall results of vaginal smear Maturation Indices in the present investigation are shown in Part 4.3. The mean range of vaginal cells represented as percentiles in the Maturation Index in all groups of females investigated prior to any oestrogen therapy was as follows :

Parabasal cells : 0 - 15.2%

Intermediate cells : 79.9 - 92.9%

Superficial cells : 3.3 - 11.1%

The mean parabasal cells in the groups with intact ovaries were 0 to 2.4%; the range for parabasal cells of the oophorectomized groups was 4.7 to 15.2%. The statistical comparison between the oophorectomized and non-oophorectomized groups proved the differences to be highly significant ($p < 0.0005$). These findings are in accordance with those of Grönroos (1965), Masukawa (1960) and Procopé (1968). Thus oophorectomy and the climacteric, representing a phase of oestrogen deficiency, has been clearly proven to be associated with a significant percentage of parabasal cells on vaginal smear. Masukawa (1960) has observed a gradual increase of parabasal cells with age and postmenopausal years.

The mean range of intermediate cells was 89.0 to 92.9% in the groups with intact ovaries and 79.9 to 84.2% in the castrated groups. Although this reflects the change in parabasal cells the statistical comparisons between these major groups showed

no differences of significance. The total proportion of intermediate cells in postmenopausal women was studied by Masukawa (1960), who obtained values of 73%, and Grönroos (1965), who reported values up to 70%. These reports, as with the present investigation, indicate intermediate cells to be predominant in all groups and both authors were unable to establish any distinct correlation with age and postmenopausal years.

The mean range of superficial cells was 6.2 to 9.8% in the groups with intact ovaries and 3.3 to 11.1% for the oophorectomized groups. These correspond roughly to the 10% arrived at by Masukawa (1960) and the 6% reported by Grönroos (1965). Thus the share of superficial cells is small and the range wide and no distinct correlation between the various groups could be established. Masukawa (1960) has shown a gradual decrease of superficial cells with age and postmenopausal years.

The Maturation Index (Frost, 1958) has been proposed by several authors in the recent medical literature as the sole indication for oestrogen treatment of postmenopausal women, a high percentage of superficial cells being held as a desirable 'norm' (Bedford, 1967; Wilson et al, 1963). For example, Bedford (1967) has reported superficial cell counts of approximately 70% for pre-climacteric females and Wilson et al (1963) have claimed that with oestrogen therapy they are able to maintain superficial cells at 80 - 85% in women aged 45 - 54. Neither author presents statistical or comparative data.

The findings of the present investigation are in conflict with those of Bedford (1967) and Wilson et al (1963). Thus, although oestrogen therapy to oophorectomized females was shown to promote the maturation of vaginal epithelial cells from the parabasal to intermediate and possibly superficial types, the only cell type to reflect a numerical change of statistical

significance was the parabasal. The mean parabasal control value was reduced from 11.54 to 0.54 after 3 months of oestradiol valerate therapy and similar significant differences were reflected in the subsequent changes to placebo therapy and again to the alternate form of therapy.

The intermediate cells reflected these changes but the statistical comparisons between the groups showed no correlations, positive or negative, of significance.

The lack of significant response by the superficial cell to oestrogen therapy, in view of reports such as those cited above, was unexpected and disappointing. The fact that 6 months' continuous oestradiol valerate therapy was unable to raise the superficial cells beyond 8.04% is considered to be a finding of significance. That this was not a property of the oestrogen administered is confirmed by the fact that 3 months' continuous high dosage conjugated oestrogen therapy resulted in a mean superficial cell value of no higher than 6.0%.

The conclusions of the present study, therefore, are that oophorectomy produces a statistically highly significant increase in the parabasal cell count ($p < 0.0005$), that this response is reversed by the administration of exogenous oestrogens, irrespective of the type used ($p < 0.0005$) and that the intermediate and superficial cells are of relatively little value in estimating the oestrogenic status of the menopausal female. For these reasons, control of oestrogen administration to the postmenopausal or oophorectomized female cannot be determined by alteration in the Maturation Index. The most reliable cytologic index is the parabasal cell.

It is therefore suggested that where oestrogen therapy is clinically indicated in postmenopausal patients the parabasal cell count expressed as a percentage of all cells present on

the vaginal smear should be used as the index of degree of response to therapy. The adoption of this suggestion would be of considerable value to the cytotechnician. As the intermediate and superficial cells may be grouped together when counting cells, a considerable amount of time could be saved in the evaluation of smears.

5.3 CHOLESTEROL METABOLISM

The current medical literature with regard to the effects of oestrogen and oophorectomy on the serum cholesterol level and the relationship of such levels to the development of atheromatosis and coronary artery thrombosis is reviewed in Part 1.4.2. The results and statistical evaluations of the serum cholesterol investigations of the present study are indicated in Part 4.4.

The results of the present investigation do not show any short-term (less than 2 years) effect of oophorectomy on the serum cholesterol. Oliver and Boyd (1959) reported a significant elevation of cholesterol in females who had undergone bilateral oophorectomy 15 to 20 years previously and a similar effect was shown for women who had undergone a spontaneous premature menopause (Sznajderman and Oliver, 1963).

Despite the limitations of direct comparison, the following summary is of interest :

DESCRIPTION	AGE RANGE	MEAN SERUM CHOLESTEROL WITH S.D.
1. Bilateral oophorectomy 15-20 years previously (Oliver and Boyd, 1959)	37-56 years	251 ± 43 mg/100 ml
2. Spontaneous premature menopause 6-20 years previously	45-49 "	299 "
Healthy women (Sznajderman and Oliver 1963)	45-49 "	217 "
3. Normal women (Barr, 1953)	45-65 "	252 "
4. Present study : Normal premenopausal	45-55 "	268 ± 43 "
Premenopausal immediately post-oophorectomy	45-55 "	250 ± 54 "
2 years post-oophorectomy	45-55 "	265 ± 41 "
2 years post-hysterectomy with conserved ovaries	45-55 "	260 ± 41 "

Thus the oophorectomized groups of Oliver and Boyd (1959) and the present investigation and the normal women of Barr's (1953) and the present investigation demonstrate remarkably similar results.

It is therefore concluded that bilateral oophorectomy in the premenopausal female does not result in an increase in the serum cholesterol for at least 2 years. This lack of early direct response to endogenous oestrogen withdrawal would therefore suggest oestrogens to be of no more than secondary importance in the known relationship of increasing blood cholesterol with age (Keys, 1963; National Centre for Health Statistics, 1966). Speculation as to what the primary factor or factors that are responsible for differences in cholesterol with age and between the sexes may be is beyond the scope of the present thesis.

The ability of exogenous oestrogen therapy to reduce serum cholesterol values appears well documented (Davis et al, 1961; Oliver and Boyd, 1961; Robinson et al, 1960). In the present study, administration of oestradiol valerate continuously for 6 months to 50 oophorectomized females resulted in a decrease of the total serum cholesterol value of possible significance ($p < 0.05$). Mixed conjugated equine oestrogen administration had no or little effect on the cholesterol values. Robinson et al (1960) reported a significant serum cholesterol lowering effect at 1 month, 3 months, 6 months and 12 months when administering conjugated oestrogens to oophorectomized females. There is no obvious explanation for the failure of the present study to confirm these findings. The present study may have been inadequste in that the period of follow-up may not have been long enough to allow a complete exhibition of all trends. A long-term study of this nature is therefore considered to be a subject for future investigation.

Nonetheless, the negative findings of the present investigation in relation to the effect of oophorectomy on serum cholesterol and the minimal short-term value of subsequent exogenous oestrogen therapy to oophorectomized females are considered sufficient evidence for questioning the empirical use of such hormones for the prevention of ischaemic heart disease in postmenopausal women (Davis et al, 1961; McBride, 1967; Wilson and Wilson, 1963; Wilson et al, 1963).

5.4 CALCIUM AND PHOSPHORUS METABOLISM

The effects of the menopause, oophorectomy and oestrogenic hormones on plasma calcium and inorganic phosphorus metabolism and their relation to osteoporosis are reviewed in Part 1.4.3. The plasma calcium, phosphorus and alkaline phosphatase values of all patients investigated in the present study are listed in Appendix D and the analysis and statistical evaluation of these results appears in Part 4.5

Whereas the present investigation does not confirm oophorectomy to result in a significant change in the plasma calcium levels, the plasma inorganic phosphorus values following oophorectomy presented some difficulty in interpretation. Phosphorus values after oophorectomy did not differ significantly from the mean normal premenopausal values. The group assessed two years after hysterectomy with conserved ovaries, however, showed a strikingly low value for plasma phosphorus which differed significantly from all the other groups investigated. These findings suggest the ovary to be effective either in producing a reduction in or preventing an elevation of plasma inorganic phosphorus and in this respect are in agreement with the findings of Szymendera and Madajewicz (1967) and Young and Nordin (1967). Young and Nordin (1967) demonstrated a significant rise in plasma and urinary calcium and phosphorus values after the menopause. In their premenopausal women, plasma calcium was 9.32 and phosphorus 3.31 mg/100 ml (9.66 and 3.61 in the present study). After the artificial menopause the corresponding values were 9.81 and 3.62 mg/100 ml (9.68 and 3.63 in the present study). They found the rise of plasma and urinary calcium to be most apparent during the first years after the cessation of the menstrual periods. These findings were confirmed by Szymendera and Madajewicz (1967).

Young and Nordin (1967) interpreted these changes to mean that the menopause is followed by a rise in bone resorption which leads to a rise in plasma and urinary phosphorus. The phosphorus values of the present investigation are similar and this interpretation is acceptable. The failure of the present investigation to confirm the changes in plasma calcium with menopause is inexplicable, especially in view of the fact that the methods of measurement were similar in both instances (Auto-Analyser) and the effect of oestrogen on plasma calcium was so dramatic in the present study.

The present investigation in fact demonstrated exogenous oestrogen therapy to be extremely effective in reducing plasma calcium and inorganic phosphorus values in oophorectomized females. These findings confirm those of Jasani et al (1965), who found that a significant fall of plasma calcium could be demonstrated following oestrogen administration provided the plasma calcium was measured by Auto-Analyser and not by flame photometry. They suggested, as a result of their study, that oestrogen hormones have an action on the blood/bone equilibrium antagonistic to the parathyroid hormone. The fall in plasma calcium and inorganic phosphorus has been shown to be due primarily to reduced bone resorption (Frost, 1961; Lafferty et al, 1964). Jasani et al (1965) calculated that, since the sites of bone loss appear to be highly selective (Nordin et al, 1966), a negative calcium balance of only 50 mg daily for five years (15 - 20 g calcium) would be sufficient to destroy a significant amount of trabecular bone. Taken into account with the findings and discussion above, the conclusion of the present investigation is that exogenous oestrogen therapy tends to lower plasma calcium and inorganic phosphorus presumably by inhibiting bone resorption. Such

therapy is therefore of potential value in the prevention of osteoporosis and is indicated, at least, in the premenopausal female who undergoes the operation of bilateral oophorectomy. These findings in relation to bone metabolism could be used as a reason for prescribing oestrogens prophylactically to all women for life from the menopause onwards.

An interesting observation of the present investigation was the disparity in effect of the two forms of oestrogen administered. Conjugated equine oestrogen was extremely effective in reducing the plasma calcium level whereas oestradiol valerate was statistically ineffective. Both oestrogens were effective in reducing plasma inorganic phosphorus, the conjugated oestrogens being slightly more effective. There are no satisfactory comparative trials in the literature. However, Young et al (1968) have shown that administration of ethinyl-oestradiol to post-menopausal women was associated with a small but significant fall in plasma calcium and phosphorus. Wallach and Henneman (1959) suggested diethylstilboestrol and conjugated equine oestrogens to be prophylactic against postmenopausal osteoporosis, their observations being clinical and radiological. Hernberg (1960) found a negligible reduction in height during ethinyl-oestradiol and hexoestrol treatment in comparison to control cases of the same age group. Neither Hernberg (1960) nor Wallach and Henneman (1959) included calcium kinetic studies in their investigations, nor did they differentiate between the specific types of oestrogen used. In both instances all cases treated were analysed together as a single group. The present investigation is therefore considered of importance in indicating that certain oestrogens are more likely to be effective than others in the future management of osteoporosis and a strict evaluation of all presently available oestrogenic substances is considered to be a subject for further research.

5.5 SPECIFIC EFFECTS ASSOCIATED WITH MENOPAUSE AND THE ROLE OF REPLACEMENT OESTROGEN THERAPY

By virtue of a critical review of the medical literature and the contribution of the present investigation, it is possible to conclude that the menopause, whether artificial or spontaneous, is associated with certain other specific clinical and metabolic effects. It has been shown, however, that the number and variety of such effects is less than currently assumed and that in certain situations these changes may be only an indirect response to oestrogen withdrawal.

It is therefore suggested that the following features are specifically related to endogenous oestrogen withdrawal, e.g. after bilateral oophorectomy :

A. CLINICAL EFFECTS :
1. Hot flushes and perspiration
2. Atrophic vaginitis - dyspareunia, pain or blood-stained discharge
3. Atrophy of breasts

B. METABOLIC EFFECTS :
1. Increase in vaginal parabasal cells
2. Increase in plasma inorganic phosphorus
3. Possible increase in plasma calcium

The increase in plasma calcium and inorganic phosphorus appears to be a result of increased bone resorption leading ultimately to clinical osteoporosis in a significant number of postmenopausal females (Young and Nordin, 1967).

The normal ovary retained at the time of gynaecological surgery in females of reproductive age is demonstrated by this study and others (Bancroft-Livingston, 1954; Beavis et al, 1969; Heller et al, 1941; Randall et al, 1957; Whitelaw, 1958) to be fully functional for at least two years after operation and probably as long as the time of the expected menopause.

The advantages have to be balanced against the risks of conservation of such ovaries, as discussed in Part 1.5. Taking all factors into account, the present study lends strength to the arguments in favour of conservation of such ovaries and the words of Robert Battey (1828-1895) bear repetition 'I believe these organs should alone be sacrificed for grave causes, and then only as a dernier resort, when the hitherto recognized resources of our art have been expended in vain.'

The specific effects of exogenous oestrogen replacement therapy have proved to be :

A. CLINICAL EFFECTS :
1. Relief of hot flushes and perspiration
2. Relief of symptoms of atrophic vaginitis (dyspareunia, pain and blood-stained discharge)
3. Produce feeling of general well-being
4. Produce nausea and vomiting

B. METABOLIC EFFECTS :
1. Reduction of vaginal parabasal cells
2. Reduction of serum cholesterol
3. Reduction of plasma calcium
4. Reduction of plasma inorganic phosphorus

The role of exogenous oestrogen replacement therapy would thus appear to have been clarified. The indications for such therapy may be enumerated as follows :

1. To postmenopausal females complaining of symptoms related to hot flushes or atrophic vaginitis
2. To all females undergoing surgical castration prior to the menopause, as a prophylactic against osteoporosis.

In this instance a case may also be made for the administration of appropriate oestrogens to all females from the menopause onwards.

The symptoms of depression, irritability, angina pectoris, insomnia and palpitations have been shown to respond adequately to placebo therapy and are not therefore indications for oestrogen administration to the postmenopausal female. Backache has been shown not to respond to oestrogen therapy. In the case of libido no effect of therapy has been demonstrated. Here the operation of hysterectomy in itself has been shown to be the factor of importance in reducing libido. Thus backache and frigidity, too, must fall away from the usual list of indications for exogenous oestrogen therapy.

The evidence relating the administration of exogenous oestrogens and the prevention of the ageing process has been examined and found to be wanting (Wilson and Wilson, 1963). For example, the present investigation has shown that these hormones are not able to improve the condition of atrophic breasts or facial appearance. There is therefore no scientific basis for such therapy at the present time (Dapunt, 1967; Novak, 1967).

Finally, the present investigation has been of value in demonstrating that the selection of oestrogen is of as much importance as the precise indication. It has been shown that different oestrogens have different effects. Thus, one particular oestrogen may be more effective than another for a specific indication and the logical conclusion is that where several specific effects are required a mixture of different oestrogens may be necessary. For example, oestradiol valerate was of value in reducing serum cholesterol and conjugated equine oestrogens were not; on the other hand, conjugated oestrogen therapy demonstrated a strong plasma calcium reducing property

after oestradiol valerate had proved to be ineffective.

The hope for the future is that oestrogenic substances will be developed with properties specific to one or other metabolic process. Thus the physician using such hormones may acquire an armamentarium of oestrogenic substances, each affecting a different target organ.

The prime objective of the present thesis has been to place the 'menopause' on a scientific basis; the scope for further investigation and advance has been shown to be considerable.

PART 6

SUMMARY AND GENERAL CONCLUSIONS

6. SUMMARY AND GENERAL CONCLUSIONS

The clinical and metabolic effects of the postmenopause have received inadequate scientific consideration. In particular, removal of normal ovaries at the time of hysterectomy on women of reproductive age has been undertaken too lightly because of insufficient knowledge or awareness of the possible disadvantageous clinical and metabolic effects of such surgery. It was thus considered that a study directed at clarifying the true clinical and metabolic effects of the menopause as produced by surgical castration, and the role of replacement oestrogen therapy, was necessary.

Accordingly, the aims of this thesis, as outlined in Part 2, were :

1. to determine the real clinical effects of bilateral oophorectomy on the human female,
2. to determine, should any clinical effects be shown, whether such effects could be reversed by exogenous oestrogen replacement therapy,
3. to study the changes in vaginal smear following bilateral oophorectomy and subsequent oestrogen therapy,
4. to study the effects of oophorectomy and subsequent oestrogen therapy on plasma cholesterol values in an identical group of patients,
5. to determine whether plasma calcium and inorganic phosphorus values were altered by oophorectomy and if so whether subsequent exogenous oestrogen therapy to the same group of patients could reverse this effect,
6. to consider the clinical and metabolic effects of bilateral oophorectomy with special reference to the place of this operation in gynaecology at the time of routine abdominal

hysterectomy for benign conditions; also to evaluate the role of replacement oestrogen therapy to all females after the menopause,

7. to determine whether different oestrogens have similar or different effects by conducting a controlled comparative evaluation of two different forms of commercially available oestrogen and a placebo.

To provide a background to these studies, relevant aspects of the literature, dealing with the historical background (Part 1.2), clinical effects of the climacteric (Part 1.3), metabolic effects of the climacteric (Part 1.4), the indications and incidence of oophorectomy (Part 1.5) and the present position of oestrogen replacement therapy (Part 1.6) were critically reviewed.

Female patients, selected according to specific criteria (Part 3.1),were investigated through a Menopause Clinic established for clinical research purposes at the Groote Schuur Hospital, Cape Town. The patients investigated were divided into 7 sub-groups according to whether they were pre- or post-menopausal and whether uterus and ovaries were intact or hysterectomy had been performed at specific time intervals, with and without removal of ovaries (Part 3.1)

Following initial evaluation of clinical features (Part 3.2.2), plasma total cholesterol, calcium, inorganic phosphorus and alkaline phosphatase (Part 3.2.3) and accurately measured vaginal smear maturation index (Part 3.2.4), the oophorectomized patients, fifty in number, received oestradiol valerate 4 mg daily continuously for 6 months (Part 3.2.5). Repeat evaluations at 3 months and 6 months preceded single blind substitution of oestradiol valerate by placebo. After 3 months' therapy, placebo was changed to 5 mg conjugated

equine oestrogen daily for 3 months. Evaluations were repeated at each substitution and following the conjugated equine oestrogen therapy. All data was recorded on special trial forms (Appendix A) and the data ultimately transferred to computer cards for the analysis and statistical evaluation (Part 3.2.6).

The following results were obtained :

1. The symptom of hot flushes and associated perspiration is shown to be oestrogen dependent - oophorectomy results in a striking increase in the incidence of hot flushes and oestrogen therapy, irrespective of type, significantly relieves the symptom ($p < 0.001$). Moreover, in this respect it is shown that the conserved ovaries are still functional two years after hysterectomy ($p < 0.01$) (Part 4.1.1.)

2. Clinical atrophic vaginitis is shown to occur commonly in the oophorectomized female and to respond dramatically to oestrogen therapy ($p < 0.001$) (Part 4.1.2.)

3. Headache is proven to be unrelated to removal of ovaries, but possibly relieved by conjugated oestrogen therapy ($p < 0.05$). Oestradiol valerate was not effective in relieving this symptom (Part 4.1.3.)

4. The response of palpitations to placebo therapy ($p < 0.05$) and the similar incidence of this symptom in all groups prior to therapy indicate that palpitations are unrelated to oophorectomy and explain the lack of response to oestrogen therapy (Part 4.1.4.)

5. Angina pectoris is shown to be a symptom related to oophorectomy ($p < 0.01$) but unrelieved by oestrogen therapy. Nevertheless, there is some relief of angina pectoris by placebo therapy ($p < 0.05$) (Part 4.1.5.)

6. The symptom of insomnia appears to be unrelated to oophorectomy, nor can it be relieved by oestrogen therapy. As expected in this situation, it demonstrates a striking response to placebo therapy ($p < 0.001$) (Part 4.1.6.)

7. Irritability is not a response of oophorectomy and is relieved essentially by placebo therapy ($p < 0.05$). Conjugated oestrogens, however, produce more relief of this symptom ($p < 0.05$) but this may be part of the general mood elevating effect of oestrogens (Part 4.1.7).

8. Observations seem to indicate that the operation of oophorectomy results in depression ($p < 0.05$), which is probably psychologically orientated in view of the highly significant response to placebo therapy ($p < 0.001$). There is no response directly attributable to oestrogen therapy (Part 4.1.8).

9. Low backache is not proven to be a factor related to oophorectomy and is not relieved by oestrogen therapy. It is considered significant that low backache, being more real a symptom than, for example, insomnia or depression, showed no placebo response to therapy (Part 4.1.9).

10. The operation of hysterectomy, irrespective of whether the ovaries are removed or not, is shown to be associated with a highly significant reduction of libido ($p < 0.001$). Oestrogen therapy has no beneficial effect on decreased or absent libido (Part 4.1.10).

11. Oestrogen and placebo therapy are both demonstrated to have a highly stimulating 'mental tonic' effect on the patient and this is borne out by both the observer's assessment and the patient's impression ($p < 0.001$). However, single blind cross-over from oestrogen to placebo resulted in a

significant deterioration in the patient's overall feeling of well-being ($p < 0.01$). In this respect both forms of oestrogen used in this trial were effective ($p < 0.01$). It is therefore considered that the 'tonic effect' of oestrogen has been proven to exist (Part 4.1.12).

12. Administration of oestrogens to oophorectomized females is shown to produce no significant change in the body weight (Part 4.2.1).

13. Oestrogen therapy does not appear to cause a change in the diastolic blood pressure (Part 4.2.2).

14. Oophorectomy results in a significant degree of breast atrophy ($p < 0.001$). However, an unexplained finding is a high incidence of atrophic breasts in the group of patients investigated 2 years after hysterectomy with conservation of ovaries as compared to a normal premenopausal group ($p < 0.001$). Moreover, exogenous oestrogen therapy is shown to be of no value in restoring atrophic breasts to a normal premenopausal state (Part 4.2.3).

15. The vaginal smear results were unexpected. The superficial cell count proved to be an unreliable guide to the oestrogenic state of the groups investigated. However, oophorectomy is shown to produce a statistically highly significant increase in the parabasal cell count ($p < 0.0005$) and this response is reversed by the administration of exogenous oestrogens, irrespective of the type used ($p < 0.0005$) (Part 4.3).

16. The present investigation does not confirm oophorectomy to result in a significant increase in the serum cholesterol. Oestrogen therapy in the form of oestradiol valerate was shown to be of value in reducing the total serum cholesterol value ($p < 0.05$) (Part 4.4).

17. Although oophorectomy did not result in any significant change in the plasma calcium value, conjugated equine oestrogen but not oestradiol valerate was shown to be extremely effective in reducing the plasma calcium ($p < 0.0025$) (Part 4.5).

18. Plasma inorganic phosphorus values after oophorectomy did not significantly differ from the mean normal premenopausal values. The 2 year post-hysterectomy-retained-ovaries group showed a strikingly low value which differed significantly from all the other groups investigated. Although these findings suggest that the ovary is effective in producing either a reduction in or preventing an elevation of plasma inorganic phosphorus, it is impossible to place any strict interpretation on these results. Nevertheless, exogenous oestrogen replacement therapy is shown to be effective in reducing plasma inorganic phosphorus and this property is common to both forms of oestrogen used (Part 4.5).

19. The only significant complication of oestrogen therapy demonstrated was a high incidence of nausea and vomiting. This effect was common to both forms of oestrogen administered and is considered to be related to the high dosage of hormone used for the purposes of this trial.

In essence, therefore, the findings in the present thesis have led to the following conclusions :

1. The only symptoms directly associated with the menopause and occurring specifically after oophorectomy are those related to hot flushes and atrophic vaginitis. In turn these symptoms are the only ones to be specifically relieved by exogenous oestrogen therapy.

2. Ovaries conserved at the time of hysterectomy in women of reproductive age have been shown to be effective in preventing postoperative menopausal symptoms for at least 2 years.

3. The symptoms of depression, irritability, angina pectoris, insomnia and palpitations respond significantly to placebo therapy and are therefore most likely of psychological origin.

4. The operation of hysterectomy, with or without conservation of ovaries, deleteriously affects libido.

5. The 'tonic effect' or ability of oestrogens to generate a feeling of well-being has been shown to exist.

6. Exogenous oestrogen has been shown to be of no value in the prevention or reversal of atrophy of the breasts of postmenopausal women.

7. Different oestrogens have different clinical and metabolic effects.

8. The percentage of vaginal parabasal cells is the most reliable cytologic index of the oestrogenic status of the post-menopausal female. Oophorectomy results in a significant increase in the number of these cells and exogenous oestrogen therapy reverses this effect. The vaginal superficial cell shows no relationship to menopause and oophorectomy, and there is a poor response by these cells following exogenous oestrogen therapy to oophorectomized women.

9. Bilateral oophorectomy in the premenopausal female does not result in an increase in the serum cholesterol for at least 2 years. Oestrogen therapy, however, reduces the serum cholesterol values of oophorectomized females and may be of possible clinical benefit.

10. Exogenous oestrogen therapy tends to lower plasma calcium and inorganic phosphorus, presumably by inhibiting bone resorption. Such therapy is therefore of potential value in the prevention of osteoporosis and is indicated, at least, in the premenopausal female who undergoes the operation of bilateral oophorectomy. By similar argument it would appear in this instance that there is some case for administration of appropriate oestrogens to all women from the menopause onwards. The present investigation, furthermore, is considered of importance in indicating that certain oestrogens are more likely to be effective than others in this respect.

11. Finally, it is concluded that ovaries of normal appearance should not be removed from women of reproductive age at the time of hysterectomy for benign indications.

PART 7

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APPENDICES

APPENDIX A

TRIAL FORM AND EXPLANATORY CODE

The trial (survey) form was designed for numerical cataloguing and assessment of each patient investigated and subsequent direct transfer to IBM 80 line computer punch cards.

The form is divided into six basic sections :

1. GENERAL : This provides for a general description of the patient and for general administrative and organizational details

2. SYMPTOMS 3. SIGNS 4. INVESTIGATIONS. These sections are self-explanatory. The numerical translation of each of the parameters measured or assessed is explained by the code sheet

5. THERAPY This section allowed for the details of treatment prescribed at each visit to be recorded

6. RESULT / RELATED DISORDERS. The measurement of factors 74 (Overall Impression), 79 (Patient's assessment) and 80 (Husband's assessment) is discussed under Section 3.2.2. The related disorders listed (items 75, 76, 77 and 78) which allow for a trial of long-term oestrogen replacement therapy and are enumerated in the explanatory code, do not apply strictly to the present thesis.

OESTROGEN REPLACEMENT SURVEY

Dr. Wulf Utian

NOT FOR CODING

Name..... Hospital No.....

History: Gynae..... General.....

Photo Taken: Face and Profile.....

CODING NUMBERS:			
GENERAL	1-3		Case Number
	4		Group Number
	5-6		Age
	7		Race
	8		Civil State
	9		Indication for Hysterectomy
	10		Previous Treatment
	11-12		Date of Visit: Day
	13-14		Month
	15		Year
	16-17		Months since Oophorectomy or Menopause
	18-19		Months on Treatment
SYMPTOMS	20		Hot Flushes
	21		Vaginal Bleeding
	22		Perspiration
	23		Headache
	24		Palpitations
	25		Angina
	26		Insomnia
	27		Mood Changes
	28		Irritability
	29		Depression
	30		Deg. Arthropathy
	31		Pruritus Valvae
	32		Dyspareunia
	33		Libido
	34		Nausea and Vomiting
	35		Allergic Reaction
	36		Miscellaneous
SIGNS	37-39		Weight (lbs.)
	40		Blood Pressure (Diastolic)
	41		Facial Hair
	42		Acne
	43		Hair Texture (Scalp)
	44-45		Skin Thickness: Subscapular
	46-47		Arm
	48-49		Height (inches)
	50		Breast Development
	51		Vaginal Discharge

A.W. LTD 53952

INVESTIGATIONS	~~52~~		~~Pap Smear~~
52-53	~~53-54~~		Superficial Cells
54-55	~~55-56~~		Intermediate Cells
56-57	~~57-58~~		Basal Cells
	~~59~~		~~Glucose Tolerance Test~~
58	~~60~~		E.C.G.
59-60	~~61~~		~~Endometrial Biopsy~~ Phosphorus (Mg/100 ml)
61	~~62~~		X-ray Lumbar Spine
62-63	~~63-64~~		Alkaline Phosphatase
64-66	~~65-66~~		Serum Calcium (Mg./100ml.)
67-69	~~67-69~~		Blood Cholesterol (Mg./100ml.)
THERAPY	70		Progynova: Dose
	71		Mode
	72		Placebo
	73		Other
RESULT	74		Overall Impression
RELATED DISORDERS	75		Myocardial Infarction
	76		Cerebral Infarction
	77		Osteoporosis
	78		Fractured Neck of Femur
	79		Patient's Assessment
	80		Husband's Assessment

NOT FOR CODING:
DISPOSAL NOTES AND MEMO:—

MENOPAUSE CLINIC

OESTROGEN REPLACEMENT CODE

4 GROUP No.

0 = NORMAL PREMENOPAUSAL
1 = PREMENOPAUSAL >45 YEARS + SYMPTOMS
2 = NORMAL POSTMENOPAUSAL
3 = POSTMENOPAUSAL + SYMPTOMS (UNOPERATED)
4 = PREMENOPAUSAL TREATED IMMEDIATE POST B.S.O.
5 = PREMENOPAUSAL TREATED 6 MONTHS " " "
6 = PREMENOPAUSAL TREATED 2 YEARS " " "
7 = POSTMENOPAUSAL TREATED IMMEDIATE POST B.S.O.
8 = 6 MONTHS POST HYSTERECTOMY - RETAINED OVARIES
9 = 2 YEARS POST HYSTERECTOMY - RETAINED OVARIES

7 RACE

0 = UNKNOWN
1 = WHITE
2 = COLOURED
3 = BANTU
4 = OTHER

8 CIVIL STATE

0 = UNKNOWN
1 = SINGLE
2 = MARRIED
3 = DIVORCED
4 = WIDOWED

9 INDICATION FOR HYSTERECTOMY

0 = UNKNOWN
1 = DYSFUNCTIONAL BLEEDING
2 = CHRONIC INFECTION
3 = BENIGN TUMOURS
4 = MALIGNANCY - CORPUS
5 = MALIGNANCY - CERVIX
6 = ENDOMETRIOSIS
7 = OTHER
8 = PROLAPSE
9 = NOT DONE

10 PREVIOUS TREATMENT

0 = UNKNOWN
1 = ANALGESIC
2 = TRANQUILIZER
3 = OESTROGEN
4 = PROGESTOGEN
5 = OEST. + TRANQ.
6 = SURGICAL
7 = NIL
8 = ANDROGEN - OESTROGEN
9 =

18-19 MONTHS ON TREATMENT

0 = PRE.OP CONTROL
1 = CONTROL
2 = 1 MONTH THERAPY
3 = 3 MONTH THERAPY
4 = 6 MONTH THERAPY
5 = 2 MONTH PLACEBO
6 = 3 MONTH PREMARIN
7 =

20 HOT FLUSHES

0 = UNKNOWN
1 = ABSENT
2 = 0-3
3 = 4-6
4 = 7-9
5 = 10-12
6 = 13-15
7 = 16-18
8 = 19-21
9 = 22 OR MORE

21-35 ALL OTHER SYMPTOMS

0 = UNKNOWN
1 = ABSENT
2 = SLIGHT
3 = MODERATE
4 = SEVERE
5 = NORMAL

27 MOOD CHANGES

0 = UNKNOWN
1 = NO CHANGE
2 = IMPROVED
3 = DETERIORATED

30 DEGENERATIVE ARTHROPATHY

0 = UNKNOWN
1 = OSTEO. OF FINGERS
2 = BACKACHE
3 = NORMAL (ABSENT)
4 = GEN. JOINT PAINS

36 MISCELLANEOUS

0 = UNKNOWN OR NIL
1 = ALLERGIC REACTION
2 = GASTRIC PAIN
3 = LOW BACK PAIN
4 = SUPERFICIAL PHLEBITIS

40 BLOOD PR.

0 = UNKNOWN
1 = 90 OR LESS
2 = 90 - 120
3 = 120 - 150
4 = 150 - 180
5 = 180 OR HIGHER

41 FACIAL HAIR & 42 ACNE

AS FOR 21 - 35

43 HAIR TEXTURE

0 = UNKNOWN
1 = NO CHANGE
2 = SLIGHT LOSS
3 = SEVERE LOSS
4 = SLIGHT GAIN
5 = EXCESS GAIN

50 BREAST DEV.

0 = UNKNOWN
1 = NORMAL PREMENOP.
2 = ATROPHIC
3 = SECRETING
4 = CANCER
5 = MASTOPATHY
6 = MASTODYNIA

51 VAG. DISCHARGE

0 = UNKNOWN
1 = ABSENT
2 = BLOOD-STAINED
3 = PHYSIOLOGIC
4 = TRICHOMONAS
5 = MONILIA
6 = NON-SPECIFIC
7 = ATROPHIC VAGINITIS
8 =

58 E.C.G.

0 = UNKNOWN
1 = NORMAL
2 = CORONARY ISCHAEMIA
3 = OLD INFARCT
4 = RECENT INFARCT
5 = HYPERTENSIVE
6 = CARDIAC ARRHYTHMIAS

61 X-RAY LUMBAR SPINE

0 = UNKNOWN
1 = NORMAL
2 = OSTEOPOROSIS
3 = VERTEBRAL COMPRESSION (COLLAPSE)
4 = OSTEO-ARTHRITIS
5 = MARKED DISC NARROWING
6 = SLIGHT DEG. CHANGES
7 = FURTHER DETERIORATION OF PATHOLOGY

70 OESTRADIOL VALERATE DOSE PER DAY

0 = UNKNOWN
1 = NIL
2 = 2 mg.
3 = 4 mg.
4 = 6 mg.
5 = 8 mg.
6 = 10 mg. or more
7 = 2 mg. every 2nd day

71 OESTRADIOL VALERATE MODE

0 = UNKNOWN
1 = CONTINUOUS
2 = CYCLICAL
3 = NIL

72 PLACEBO

0 = UNKNOWN
1 = CONTINUOUS
2 = CYCLICAL

73 OTHER

0 = UNKNOWN
1 = NIL
2 = ANALGESIC
3 = ANOREXIC
4 = TRANQUILIZER
5 = 2.5 mg conj. oestrogen
6 = 1.25 mg conj. oestrogen
7 = 5.0 mg conj. oestrogen
8 = 10.0 mg conj. oestrogen

75-78 RELATED DISORDERS

0 = UNKNOWN
1 = ABSENT
2 = BEFORE TREATMENT
3 = DURING TREATMENT
4 = AFTER TREATMENT

74 RESULT 79 PATIENT & 80 HUSBAND

0 = UNKNOWN
1 = MARKED IMPROVEMENT
2 = SLIGHT IMPROVEMENT
3 = UNCHANGED
4 = SLIGHT DETERIORATION
5 = MARKED DETERIORATION

75 - 5 = PREVIOUS DEEP VEIN THROMBOSIS

APPENDIX B

VAGINAL EPITHELIAL CELL COUNTS OF ALL PATIENTS AT EACH ASSESSMENT REPRESENTED AS MATURATION INDICES (PARABASAL : INTERMEDIATE : SUPERFICIAL CELL TYPES)

TABLE B.I

CELL COUNTS OF ALL PATIENTS AT EACH VISIT REPRESENTED AS MATURATION INDICES VIZ :
PARABASAL : INTERMEDIATE : SUPERFICIAL CELLS

PATIENT CLINIC NUMBER	CONTROL			3 MONTH OESTRADIOL VALERATE THERAPY			6 MONTH OESTRADIOL VALERATE THERAPY			3 MONTH PLACEBO THERAPY			3 MONTH CONJUGATED OESTROGEN THERAPY		
	PARAB.	INT.	SUP.	PARAB.	INT.	SUP.	PARAB.	INT.	SUP.	PARAB.	INT.	SUP.	PARAB.	INT.	SUP.
04	04	77	19	00	97	03	00	99	01	00	99	01	00	98	02
07	01	98	01	00	92	08	00	98	02	00	99	01	00	98	02
08	50	49	01	00	99	01	00	94	06	45	55	00	00	90	10
09	06	87	07	00	98	02	00	99	01	01	99	00	01	98	01
10	00	92	08	00	97	03	00	98	02	04	90	06	00	93	07
11	32	66	02	00	87	13	00	93	07	00	97	03	01	97	02
12	00	99	01	00	80	20	25	75	00	21	77	02	03	97	00
13	00	99	01	00	87	13	00	90	10	05	94	01	00	91	09
14	31	63	06	00	99	01	00	99	01	00	99	01	00	98	02
16	02	96	02	00	95	05	00	99	01	00	99	01	00	99	01
17	00	83	17	00	83	17	00	78	22	00	75	25	00	55	45
18	33	67	00	04	95	01	20	80	00	04	96	00	01	99	00
19	01	77	22	00	93	07	02	96	02	02	98	00	00	98	02
20	01	99	00	00	97	03	00	90	10	01	99	00	00	90	10
22	00	95	05	01	99	00	00	97	03	00	95	05	00	97	03
24	15	78	07	00	93	07	00	99	01	03	97	00	00	98	02
25	01	99	00	00	99	01	00	92	08	00	99	01	00	85	15
26	00	65	35	00	98	02	00	99	01	60	40	00	00	99	01
27	10	90	00	00	95	05	01	98	01	02	98	00	00	98	02
29	00	93	07	01	98	01	00	97	03	00	99	01	-	-	-
30	00	99	01	00	95	05	00	99	01	01	98	01	00	92	08
31	00	80	20	01	99	00	00	99	01	00	99	01	00	99	01

33	01	99	00	00	96	04	00	99	01	00	99	01	00	95	05
35	10	90	00	08	90	02	07	93	00	25	75	00	01	99	00
36	00	95	05	00	87	13	00	91	09	05	90	05	02	96	02
38	45	55	00	00	99	01	00	99	01	03	97	00	00	99	01
40	00	87	13	00	94	06	00	96	04	01	99	00	01	99	00
41	00	96	04	00	90	10	00	90	10	01	99	00	00	99	01
43	13	85	02	01	97	02	00	85	15	00	93	07	04	93	03
44	20	80	00	01	99	00	00	97	03	75	25	00	01	95	04
47	02	97	01	00	95	05	00	98	02	61	99	00	00	99	01
48	22	78	00	02	98	00	00	98	02	00	99	01	05	93	02
49	00	97	03	00	99	01	00	99	01	00	99	01	00	99	01
50	01	82	17	00	99	01	00	75	25	00	99	01	02	97	01
51	00	40	60	00	60	40	00	45	55	25	75	00	00	50	50
52	50	50	00	04	96	00	00	70	30	08	90	02	00	80	20
53	00	99	01	02	93	05	00	94	06	00	99	01	05	95	00
54	00	94	06	00	90	10	00	97	03	00	96	04	01	91	08
55	70	30	00	00	75	25	00	77	23	00	99	01	00	93	07
56	00	85	15	00	75	25	00	60	40	02	89	09	00	78	22
57	35	65	00	00	75	25	00	93	07	03	97	00	00	90	10
58	00	96	04	00	98	02	00	99	01	01	99	00	01	99	00
59	60	40	00	00	85	15	00	94	06	15	85	00	00	96	04
62	00	92	08	00	99	01	00	99	01	01	96	03	04	95	01
71	01	97	02	00	95	05	01	87	12	39	60	01	00	93	07
72	05	70	25	01	99	00	00	99	01	04	96	00	01	98	01
73	01	98	01	01	99	00	00	96	04	55	44	01	00	97	03
81	50	50	00	00	83	17	00	84	16	04	93	03	00	93	07
91	03	96	01	00	80	20	00	90	10	00	90	10	00	98	02
93	00	91	09	00	80	20	00	70	30	00	93	07	01	93	06
TOTAL PATIENTS		50			50			50			50			49	

TABLE B.2 CELL COUNTS REPRESENTED AS MATURATION INDICES OF NORMAL PREMENOPAUSAL GROUP AND GROUPS WITH CONSERVED OVARIES AFTER HYSTERECTOMY

GROUP DESCRIPTION	PATIENT CLINIC NUMBER	PARABASAL	INTERMEDIATE	SUPERFICIAL
Normal premenopausal (9 patients)	31	0	99	1
	32	0	97	3
	55	1	99	0
	60	0	75	25
	71	0	70	30
	105	0	92	8
	106	10	85	5
	107	0	99	1
	108	0	85	15
6 months post-hysterectomy with conserved ovaries (8 patients)	3	0	90	10
	15	0	99	1
	79	0	95	5
	85	0	98	2
	90	0	99	1
	96	0	85	15
	101	20	80	0
	103	0	97	3
2 years post-hysterectomy with conserved ovaries (18 patients)	1	0	92	8
	63	0	98	2
	64	15	80	5
	65	0	99	1
	66	0	99	1
	67	0	99	1
	75	0	50	50
	76	0	96	4
	78	1	99	0
	80	1	82	17
	83	0	97	3
	84	0	95	5
	89	1	99	0
	94	4	93	3
	97	2	96	2
	98	0	97	3
	101	20	80	0
	104	0	93	7

APPENDIX C

BLOOD CHOLESTEROL ESTIMATIONS OF ALL PATIENTS INVESTIGATED

TABLE C.I BLOOD CHOLESTEROL VALUES IN MG PER 100 ML OF ALL OOPHORECTOMIZED PATIENTS AT EACH VISIT

TABLE C.2 BLOOD CHOLESTEROL VALUES OF NORMAL PREMENOPAUSAL GROUP AND GROUPS WITH CONSERVED OVARIES AFTER HYSTERECTOMY

TABLE C.I BLOOD CHOLESTEROL VALUES (MG PER 100 ML) OF ALL OOPHORECTOMIZED PATIENTS AT EACH VISIT

GROUP DESCRIPTION	PATIENT CLINIC NUMBER	CONTROL	3 MONTH OESTRADIOL VALERATE	6 MONTH OESTRADIOL VALERATE	3 MONTH PLACEBO	3 MONTH CONJUGATED OESTROGEN
Premenopausal - investigation commenced immediately post-oophorectomy	4	204	200	239	212	196
	7	204	220	242	210	265
	8	389	293	292	296	235
	9	220	203	226	226	220
	10	202	230	218	258	227
	13	270	259	252	297	249
	14	228	175	218	232	206
	17	209	225	214	200	148
	31	243	211	194	207	219
	55	334	302	293	266	292
	71	210	262	252	268	238
	72	278	286	237	293	287
	73	250	225	191	264	230
	91	266	313	263	318	257
Premenopausal - investigation commenced 6 months post-oophorectomy	22	194	215	189	181	181
	24	207	260	207	243	262
	26	300	293	293	326	290
	30	241	243	175	204	226
	35	290	242	247	253	268
	41	232	260	220	432	379
	43	253	230	248	278	308
	44	223	207	256	217	226
	47	247	247	242	215	234
	49	273	244	259	273	280
	50	285	257	243	243	268
	51	254	254	259	277	218
	93	262	250	280	266	273

Premenopausal - investigation commenced 2 years post-oophorectomy	12	269	270	220	262	233
	25	303	260	239	313	290
	27	303	280	264	273	302
	29	281	220	197	222	-
	33	332	286	280	308	262
	36	296	183	231	231	252
	38	230	257	257	248	315
	40	263	286	263	274	330
	48	219	203	204	207	216
	52	297	255	280	307	284
	53	260	260	244	260	308
	54	318	246	265	354	268
	56	182	164	185	200	193
	57	293	229	247	304	252
	58	229	218	200	239	237
	59	251	243	258	281	252
	62	200	189	207	200	220
	81	252	207	247	248	266
Postmenopausal - investigation commenced immediately post-oophorectomy	11	293	254	261	257	260
	16	265	243	268	276	261
	18	228	286	347	296	289
	19	253	357	289	313	281
	20	169	220	200	158	190

TABLE C.2 BLOOD CHOLESTEROL VALUES (MG PER 100 ML) OF NORMAL PREMENOPAUSAL GROUP AND GROUPS WITH CONSERVED OVARIES AFTER HYSTERECTOMY

GROUP DESCRIPTION	PATIENT CLINIC NUMBER	CHOLESTEROL
Normal premenopausal	31	227
	32	311
	55	334
	60	242
	71	241
	105	230
	106	257
	107	330
	108	245
6 months post-hysterectomy with conserved ovaries	3	243
	15	191
	79	235
	85	207
	90	274
	96	276
	101	300
	103	254
2 years post-hysterectomy with conserved ovaries	1	251
	63	263
	64	270
	65	261
	66	270
	67	233
	75	346
	76	320
	78	272
	80	253
	83	228
	84	280
	89	193
	94	309
	97	223
	98	215
	101	300
	104	200

APPENDIX D

PLASMA CALCIUM, PHOSPHORUS AND ALKALINE PHOSPHATASE VALUES OF ALL PATIENTS INVESTIGATED

TABLE D.I PLASMA CALCIUM VALUES, IN MG PER 100 ML, OF ALL OOPHORECTOMIZED PATIENTS AT EACH VISIT

GROUP DESCRIPTION	PATIENT CLINIC NUMBER	CONTROL	3 MONTH OESTRADIOL VALERATE	6 MONTH OESTRADIOL VALERATE	3 MONTH PLACEBO	3 MONTH CONJUGATED OESTROGEN
Premenopausal - investigation commenced immediately post-oophorectomy	4	9.6	8.5	8.9	9.6	9.5
	7	9.4	9.8	9.4	8.9	8.8
	8	9.6	9.7	9.7	9.4	9.5
	9	9.8	9.4	9.2	9.2	8.2
	10	9.0	9.0	9.3	9.8	9.0
	13	9.4	9.4	10.1	10.5	9.3
	14	9.3	8.4	10.5	10.2	9.8
	17	10.0	10.4	10.0	11.1	9.5
	31	10.0	9.2	9.2	9.1	8.1
	55	9.6	9.5	8.5	9.7	9.4
	71	9.0	10.3	10.3	9.2	8.7
	72	10.0	10.2	9.4	9.6	8.9
	73	10.5	8.9	9.4	9.2	9.0
	91	9.7	10.3	9.7	8.5	8.3
Premenopausal - investigation commenced 6 months post-oophorectomy	22	9.7	9.9	8.6	10.3	8.8
	24	9.2	10.2	9.8	9.1	10.3
	26	10.0	9.6	10.2	10.4	10.2
	30	10.0	9.5	8.7	9.5	9.0
	35	8.7	9.2	8.8	9.4	9.0
	41	7.0	9.8	9.0	9.6	9.2
	43	10.4	9.5	9.2	9.6	9.2
	44	9.2	9.3	9.3	9.2	10.0
	47	9.0	9.0	9.3	9.7	8.9
	49	10.0	9.8	9.9	9.6	9.9
	50	10.3	9.8	8.5	9.4	9.3
	51	10.3	9.8	10.1	9.7	10.6
	93	9.5	9.7	11.3	9.9	9.1

Premenopausal - investigation commenced 2 years post-oophorectomy	12	8.9	7.6	9.7	10.2	10.2
	25	9.8	9.0	9.4	8.3	9.1
	27	10.8	9.6	9.4	10.3	9.2
	29	10.4	9.3	9.3	8.7	-
	33	10.8	10.2	10.5	9.0	9.9
	36	9.7	9.6	10.2	10.2	9.1
	38	9.8	10.4	10.5	9.7	10.4
	40	9.6	9.2	9.2	10.6	9.7
	48	8.3	9.4	9.6	10.0	8.5
	52	10.4	10.4	10.7	9.8	11.3
	53	10.3	9.3	9.8	9.0	8.4
	54	10.2	9.4	9.7	9.3	8.2
	56	9.1	9.3	9.3	9.2	8.6
	57	9.0	10.6	10.6	9.5	8.9
	58	10.6	10.1	9.6	9.3	9.4
	59	9.4	9.3	9.2	9.0	8.3
	62	9.9	10.1	9.9	9.2	9.2
	81	9.4	8.5	8.5	9.1	8.6
Postmenopausal - investigation commenced immediately post-oophorectomy	11	10.1	8.5	9.6	9.4	8.8
	16	9.8	9.6	10.4	9.4	9.0
	18	10.0	9.9	9.8	9.3	9.6
	19	9.6	10.5	9.9	9.7	10.0
	20	9.9	9.2	8.6	9.7	9.1

TABLE D.2 PLASMA INORGANIC PHOSPHORUS VALUES, IN MG PER 100 ML, OF ALL OOPHORECTOMIZED PATIENTS AT EACH VISIT

GROUP DESCRIPTION	PATIENT CLINIC NUMBER	CONTROL	3 MONTH OESTRADIOL VALERATE	6 MONTH OESTRADIOL VALERATE	3 MONTH PLACEBO	3 MONTH CONJUGATED OESTROGEN
Premenopausal - investigation commenced immediately post-oophorectomy	4	4.2	3.9	5.2	4.2	3.3
	7	3.4	3.8	3.3	4.1	3.3
	8	3.0	2.2	2.0	2.8	2.6
	9	4.0	3.0	3.2	3.0	4.1
	10	3.0	3.0	3.2	4.3	2.8
	13	3.6	3.3	3.1	3.9	2.6
	14	3.7	3.8	3.2	3.7	3.3
	17	3.9	3.6	3.2	3.4	2.8
	31	4.6	3.2	3.7	3.2	3.1
	55	3.9	3.5	3.7	3.1	3.6
	71	4.7	3.8	2.6	3.5	2.8
	72	4.4	3.2	3.5	3.1	2.6
	73	4.1	3.4	3.1	4.1	3.5
	91	4.5	6.2	3.9	5.1	3.1
Premenopausal - investigation commenced 6 months post-oophorectomy	22	4.1	2.6	3.0	3.2	3.4
	24	2.9	3.3	2.8	4.2	3.1
	26	3.4	3.4	3.8	3.7	3.7
	30	2.6	3.9	4.5	2.6	3.2
	35	4.2	3.7	4.9	3.2	3.5
	41	3.1	2.8	4.2	4.4	2.1
	43	3.6	3.1	3.3	3.5	3.7
	44	3.8	3.2	3.4	3.9	3.1
	47	3.0	3.0	4.1	5.3	3.5
	49	3.6	3.3	3.1	3.6	3.1
	50	4.1	3.0	4.1	2.8	3.4
	51	3.8	3.5	3.9	4.5	3.1
	93	3.7	3.7	3.7	3.6	3.8

Premenopausal - investigation commenced 2 years post-oophorectomy	12	3.2	2.5	3.1	2.7	2.7
	25	3.5	3.2	2.7	3.7	3.7
	27	3.1	2.8	2.9	2.6	2.7
	29	3.7	3.7	3.8	4.7	-
	33	3.6	3.0	3.2	3.7	3.2
	36	3.9	3.6	3.9	3.9	4.4
	38	3.5	3.5	3.6	3.6	2.3
	40	3.0	3.3	3.3	3.9	3.0
	48	4.1	3.2	3.8	2.8	3.8
	52	3.9	4.1	3.9	3.4	3.4
	53	3.9	3.0	2.3	2.7	2.8
	54	4.0	3.2	3.2	4.0	3.4
	56	3.5	3.4	3.3	3.5	3.1
	57	2.5	3.5	3.5	4.0	3.2
	58	3.7	3.5	4.4	4.0	3.7
	59	3.3	3.5	4.0	3.8	3.5
	62	2.8	3.3	4.1	4.0	5.1
	81	3.1	3.5	2.1	2.9	2.9
Postmenopausal - investigation commenced immediately post-oophorectomy	11	3.7	3.8	3.7	3.7	4.8
	16	3.3	4.7	4.8	2.7	2.9
	18	4.2	2.5	3.8	4.1	3.6
	19	3.2	2.9	2.7	3.4	2.4
	20	3.9	2.4	2.7	3.2	1.9

TABLE D.3 PLASMA ALKALINE PHOSPHATASE VALUES, IN BODANSKY-REINHART UNITS, OF ALL OOPHORECTOMIZED PATIENTS AT EACH VISIT

GROUP DESCRIPTION	PATIENT CLINIC NUMBER	CONTROL	3 MONTH OESTRADIOL VALERATE	6 MONTH OESTRADIOL VALERATE	3 MONTH PLACEBO	3 MONTH CONJUGATED OESTROGEN
Premenopausal - investigation commenced immediately post-oophorectomy	4	2.1	4.0	3.2	1.2	1.6
	7	2.1	2.4	2.1	1.7	2.3
	8	3.5	1.2	2.6	3.0	2.4
	9	2.3	2.4	0.6	0.6	2.0
	10	1.5	1.2	2.4	2.6	3.0
	13	4.2	6.2	3.4	2.1	3.5
	14	2.8	2.0	1.5	1.5	2.9
	17	5.6	2.8	1.5	2.0	2.4
	31	2.7	0.9	2.5	2.6	3.3
	55	2.8	2.1	2.8	4.1	3.4
	71	3.5	3.6	3.0	3.2	3.5
	72	3.8	1.4	3.0	2.9	3.1
	73	3.4	2.0	4.2	4.0	3.4
	91	1.6	2.0	6.4	4.9	4.2
Premenopausal - investigation commenced 6 months post-oophorectomy	22	4.7	2.2	2.9	3.4	4.0
	24	3.0	9.0	2.7	3.8	1.4
	26	3.2	1.0	1.7	3.0	1.4
	30	2.1	1.2	3.2	3.6	3.8
	35	3.3	2.2	3.2	3.7	4.8
	41	3.8	2.1	2.9	7.5	6.1
	43	3.9	1.7	2.9	3.5	4.2
	44	4.4	2.2	2.8	4.0	1.2
	47	4.5	4.5	4.8	5.6	5.8
	49	3.6	2.1	2.6	3.0	1.0
	50	3.6	2.8	4.1	3.6	3.5
	51	2.3	2.0	2.9	2.5	1.0
	93	2.7	4.7	7.6	2.9	5.2

Premenopausal - investigation commenced 2 years post-oophorectomy	12	3.2	2.1	2.1	2.3	3.0
	25	3.9	1.2	2.8	3.0	3.6
	27	6.2	1.1	2.2	3.8	1.7
	29	5.0	3.9	4.4	4.3	-
	33	3.6	2.1	2.9	2.6	1.2
	36	6.5	2.8	3.3	3.3	4.3
	38	2.7	2.0	2.3	2.8	3.2
	40	2.7	2.6	2.1	3.5	1.9
	48	2.7	2.1	3.1	2.8	2.5
	52	3.1	2.0	2.4	2.9	1.3
	53	1.5	1.5	3.3	3.7	4.0
	54	2.5	3.0	1.8	3.8	3.4
	56	3.0	1.5	2.5	2.8	3.0
	57	1.1	1.1	1.5	2.7	1.9
	58	4.2	2.0	4.5	5.6	5.0
	59	3.7	2.8	3.9	3.1	4.1
	62	2.6	1.9	2.6	3.5	3.9
	81	1.7	1.3	2.9	3.6	3.2
Postmenopausal - investigation commenced immediately post-oophorectomy	11	2.7	3.7	3.8	3.0	3.5
	16	8.1	4.3	2.1	4.4	3.4
	18	4.2	4.1	2.3	4.5	6.0
	19	3.2	3.3	1.7	1.4	3.1
	20	4.7	3.5	3.6	3.9	3.9

TABLE D.4 PLASMA CALCIUM, INORGANIC PHOSPHORUS AND ALKALINE PHOSPHATASE VALUES OF THE NORMAL PREMENOPAUSAL GROUP AND THE GROUPS WITH CONSERVED OVARIES AFTER HYSTERECTOMY

GROUP DESCRIPTION	PATIENT CLINIC NUMBER	CALCIUM Mg per 100 ml	PHOS. Mg per 100 ml	ALK. PHOS. B.-R. Units
Normal premenopausal	31	9.3	4.3	2.7
	32	9.9	4.2	2.4
	55	9.5	3.6	4.5
	60	9.4	3.6	3.4
	71	9.8	3.7	3.3
	105	9.8	3.6	3.4
	106	8.7	3.5	3.0
	107	9.7	2.6	1.0
	108	10.8	3.4	3.3
6 months post hysterectomy with conserved ovaries	3	9.7	3.0	3.1
	15	9.2	4.8	2.8
	79	10.3	3.1	4.7
	85	9.3	4.0	1.5
	90	9.9	3.4	3.5
	96	9.9	4.8	1.7
	101	10.0	3.4	1.0
	103	9.9	2.7	1.8

2 years post hysterectomy with conserved ovaries	1	9.0	3.0	2.3
	63	9.7	2.0	3.7
	64	9.7	2.6	6.3
	65	9.9	3.3	4.6
	66	9.7	2.7	3.1
	67	9.6	3.0	5.5
	75	8.7	3.0	3.6
	76	8.8	2.6	4.5
	78	10.0	2.6	5.3
	80	10.0	3.2	0.9
	83	10.0	4.0	1.7
	84	10.2	2.4	1.8
	89	9.9	3.0	1.3
	94	10.0	2.6	1.2
	97	9.1	3.0	1.5
	98	9.3	3.7	1.4
	101	10.0	3.4	1.0
	104	10.0	3.7	1.5